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The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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ACCEPTANCE OF MANUSCRIPTS

The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to 12-double-spaced typed pages, including tables, graphs, and photographs. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases. By following this procedure we hope to continue publishing all articles promptly.

Paul R. Miller

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IN THIS ISSUE

VIRUS

C. E. YARWOOD found that soaking bean leaves in water before inoculation increased infection by several different viruses, but he concluded that at present the results of experiments with different water effects in virus inoculations cannot be reconciled either with one another or with what is known about darkness effects, diurnal effects, and carbohydrate effects, page 841.

In experiments conducted by JAMES D. PANZER, relative himidity was shown to influence infection of local lesion test plants by certain viruses, but atmospheric pressure had no effect, page 845.

J. T. SLYKHUIS, F. J. ZILLINSKY, A. E. HANNAH, and W. R. RICHARDS recount investigations on distribution, vector relations, effect of time of infection, and other aspects of barley yellow dwarf virus occurrence in cereal crops in Ontario, page 849.

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B. F. LOWNSBERY, JOHN T. MITCHELL, and ERNEST E. HEAD describe an electric auger for taking soil samples, page 918.

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REINER BONDE, ROBERT AKELEY, and DONALD MERRIAM report that some desirable potato seedlings resistant to bacterial ring rot were also found to be highly resistant to or immune from one or more races of the late blight fungus, page 924.

OTIS C. MALOY states that in his studies of microbial associations in Fusarium root rot of bean, organisms determined by microscopic examination to be present in the roots were generally different in type from organisms isolated in culture, page 929.

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WALTER H. BURKHOLDER discusses the relation of several different species of bacteria to the black stem disease of annual larkspur, page 934.

JAMES D. BILBRUCK, on the basis of a series of chemical reactions, considers that the substance responsible for the toxicity of oak heartwood to the oak wilt fungus is probably tartaric acid, page 936.

E. RICHARD TOOLE and R. C. MORRIS report evidence indicating that an undetermined fungus is the cause of a trunk lesion of sweetgum trees in the South, page 942.

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JOHN P. JONES and EDGAR E. HARTWIG describe a simplified method for field inoculation of soybeans with the bacterial pustule organism, which would probably be useful for field use with other leaf-infecting bacteria also, page 946.

J. B. SINCLAIR, N. L. HORN, and W. J. MARTIN report some unusual disease occurrences recently observed in Louisiana, page 947.

Unusual Records on Plant Diseases, page 949: Unusual occurrence of tomato leaf mold in Louisiana, by J. B. SINCLAIR.

Corrections, page 949.

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CONTRIBUTORS PLEASE NOTE REVISION IN "ACCEPTANCE OF MANUSCRIPTS" PARAGRAPH ON PAGE 838 (INSIDE FRONT COVER).

VIRUS SUSCEPTIBILITY INCREASED BY SOAKING BEAN LEAVES IN WATER

C. E. Yarwood

Abstract

When the primary leaves of Pinto bean were immersed in water for several hours, and then inoculated with tobacco virus, tobacco necrosis virus, peach yellow bud mosaic virus, or apple mosaic virus the number of local lesions resulting was usually greater than the number on comparable leaves which had not been soaked in water. When the immersion was started at 4 p.m. to 8 p.m., the susceptibility of the treated leaves was less than that of the control leaves for the next 14 hours, but then rose to a much higher degree of susceptibility than the controls. The greatest average increase in infection (thirteen fold for four replications) was for inoculations made at 4 a.m. to 5 a.m. on leaves which had been in water the previous 34 hours. The increased susceptibility due to soaking leaves in water was much less when the inoculum was suspended in 1 percent K_2HPO_4 than when suspended in water. No increased susceptibility was observed from soaking cucumber cotyledons in water before inoculating them with peach ring spot virus.

METHODS

Most studies were with tobacco mosaic virus (TMV) on Pinto bean (Phaseolus vulgaris). One primary leaf of each plant, 9 to 15 days from seeding, was immersed in water at greenhouse temperatures; the opposite leaf served as a control. For convenience, beans in 4-inch pots were laid horizontally, and one leaf was immersed vertically in a jar of water. For immersion periods greater than 3 hours there was a strong tendency for the immersed leaf to turn up out of the water; a bent glass rod was rested on the immersed leaf to hold it in place. After the specified immersion period, the immersed leaf was removed from the water, quickly dried with an air blast, and the control leaf and immersed leaf were inoculated with a 0.1 percent water suspension of macerated infected tobacco leaves. In most cases, one-half of each leaf was quickly dried after inoculation and the other half was allowed to dry naturally in the greenhouse. Lesions were counted about 5 days after inoculation. The number of lesions on the soaked leaf was expressed as a decimal fraction of the number on the control leaf to give an infection quotient. Infection quotients less than 1 indicate that soaking in water reduced infection, whereas quotients greater than 1 indicate that soaking in water increased susceptibility. For example, one leaf placed in water at 4 p.m., April 17, and inoculated at 8 p.m., April 18, yielded 376 lesions on the quick-dried half of the leaf, and 51 lesions on the half of the leaf allowed to dry naturally. The opposite unsoaked control yielded 18 lesions on the quick-dried and 6 lesions on the natural-dried halves. The quotients for the water-soaking effect were, therefore, 376/18 = 20.8 for the quick-dried and 51/6 = 8.5 for the natural-dried leaves. The quotient for quick drying was 376/51 = 7.4 for the soaked leaf and 18/6 = 3 for the control leaf.

The principal additional variables introduced were time of start of soaking, duration of soaking, effect of phosphate in the inoculum, different viruses, and different hosts.

Greenhouse temperatures, which were not studied, but which may be important in the response of leaves to soaking in water, usually ranged from a minimum of about 15° C at night to a maximum of about 27° during the day.

RESULTS, TOBACCO MOSAIC VIRUS

In an initial trial, soaking of different leaves was started at 6 a.m., 10 a.m., 2 p.m., 6 p.m., and 10 p.m., with inoculation about 4 or 8 hours later. Increased infection resulted for each period of soaking except that started at 6 p.m., and the greatest increase resulted from starting of soaking at 6 a.m. In most later trials, soaking was started at 4 a.m. to 8 a.m. or 4 p.m. to 6 p.m. All results for soakings started during these periods, and under normal greenhouse temperatures are summarized in Figure 1.

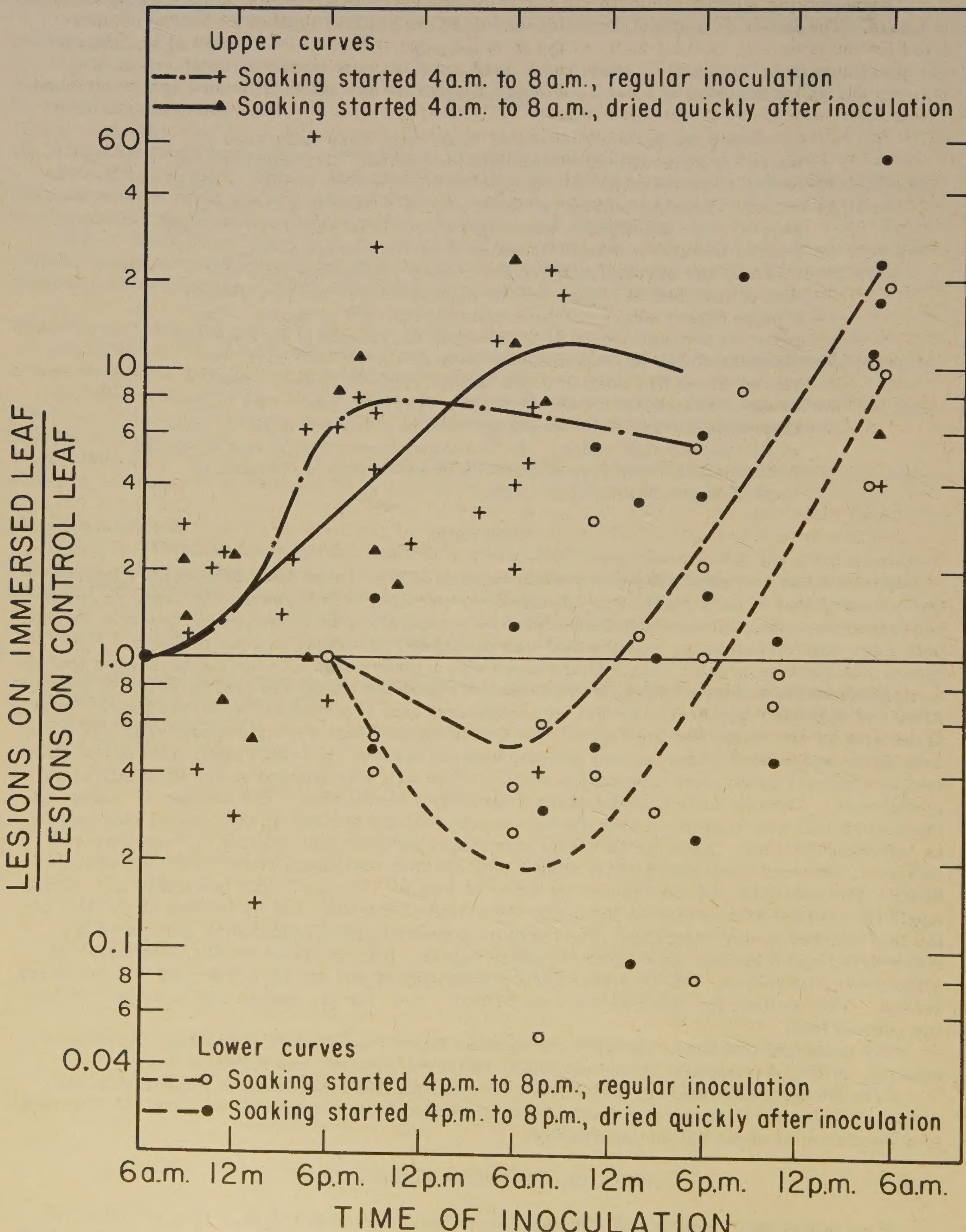

FIGURE 1. The effect of duration of immersion in water on the subsequent susceptibility of bean leaves to tobacco mosaic virus.

Water-soaking started at 4 a.m. to 8 a.m. increased susceptibility to TMV in 36 out of 43 trials. The infection quotient for quick-dried leaves appeared greater than for normal-dried leaves from 4 p.m. to 8 p.m. of the first day, but it is not clear if this is significant. The maximum infection quotient observed was 63 for a leaf immersed in water at 7 a.m., January 24, and inoculated at 5 p.m., January 24, but no special significance can be attached to this high value since it was not replicated or repeated. The nine infection quotients above 10 all fell in the period (5 p.m. to 9 a.m.) where inoculation was 11 to 27 hours after the start of water-soaking, and for this period the arithmetic average of all infection quotients was 11.5. This arithmetic average gives an infection quotient greater than is real, since an arithmetic average gives too little weight to the low values. The arithmetic average derived from the logarithms of the infection quotients is 6.3, which is a truer picture of the effect of water-soaking on the susceptibility of bean leaves to TMV.

Water-soaking started at 4 p.m. to 8 p.m. decreased susceptibility if the leaves were inoculated within about 24 hours after the start of water-soaking, but increased susceptibility if the leaves were inoculated between about 24 and 36 hours after the start of soaking. For all inoculations made from 34 to 36 hours after the start of soaking, the average infection quotient was 26 for four inoculations made with quick drying, and 11 for inoculations followed by normal drying. The greater infection quotients associated with quick drying than with normal drying appeared highly significant for soakings started at 4 p.m. to 8 p.m. but of questionable significance for soakings started at 4 a.m. to 8 a.m.

OTHER VIRUSES

When Pinto bean leaves were soaked in water from 6:40 a.m., May 14, to 8 p.m., May 14, and then inoculated, the infection quotients for numbers of lesions due to soaking the leaves in water were: 1.9 for one strain of TNV, 1.4 for another distinct strain of TNV, 2.0 for peach yellow bud mosaic, and 9.0 for apple mosaic virus. These limited results indicate that these three viruses respond to pre-inoculation soaking of the suscept in a similar way to TMV.

OTHER HOSTS

Cucumber was the only host tested in addition to bean. When cucumber cotyledons were soaked in water from 9 a.m. to 5 p.m., May 21, in one test and 7 a.m. to 5 p.m., May 22, in another test and then inoculated with peach ring spot virus, the infection quotients were 0.3 and 0.7, respectively.

PHOSPHATE IN THE INOCULUM

When bean leaves were soaked in water from 5:30 a.m., April 18, to 5:30 a.m., April 19, and then inoculated with 0.001 percent TMV + 1 percent K_2HPO_4, the infection quotients due to soaking in water were 1.4 for the quick-dried and 2.0 for the regular-dried leaves. In the same test, the infection quotients for leaves inoculated without phosphate were 24.4 for the quick-dried and 12.3 for the regular-dried. In another trial, the infection quotients were 5.0 for leaves inoculated with phosphate and 16.6 for leaves inoculated without phosphate. The addition of phosphate to the inoculum, therefore, reduces but does not eliminate the increased susceptibility due to soaking the leaves in water.

The lower response from water-soaking when phosphate was used in the inoculum indicates, of course, that the direct response to phosphate was lower on leaves that had been soaked in water than on unsoaked leaves. In the case cited above for leaves inoculated at 5:30 a.m., April 19, the infection quotient due to phosphate $\frac{\text{(lesions on leaves inoculated with phosphate)}}{\text{(lesions on leaves inoculated without phosphate)}}$ was 835 for control of unsoaked leaves and 65 for water-soaked leaves.

DISCUSSION

Predisposition of leaves to virus susceptibility by soaking them in water is only one of many ways of modifying virus transmission (5). Among prior treatments it seems most closely related to predisposition by high soil moisture as reported by Tinsley (3). As yet it has been shown to apply only to beans and with beans there is no good reason to believe it has general utility. Major objections to its use are the long treatment periods necessary, which are awkward to apply. Furthermore, the addition of phosphate to the inoculum is a simpler

and more effective way of increasing the susceptibility of beans to several viruses. When phosphate was used in the inoculum, the effect of pre-inoculation soaking of the leaves was much less than when no phosphate was used, so the two treatments are not additive in effect. Soaking the leaves in aqueous phosphate has not been so effective as adding phosphate to the inoculum.

Predisposition by water-soaking seems of interest primarily because of its relation to other predisposing treatments used in virus transmission. Heat treatments to increase susceptibility to virus have been studied by heating the leaves in water (4) and the heat effect could have been partly a water effect. The increased susceptibility due to holding leaves in the dark before inoculation with viruses (2) may be in part a water effect, as leaves in the dark would normally be at a higher humidity than leaves in sunlight. If that were the case, however, one would expect that normally exposed plants would be more susceptible to virus at night or in the early morning after a dark period than in the afternoon. Reports by Matthews (1) and Yarwood (5) indicate the reverse. The water-soaking effect may itself be diurnal in that immersions started at 4 a.m. to 4 p.m. usually resulted in an initial increase in susceptibility, whereas immersions started at 4 p.m. to 8 p.m. usually resulted in an initial decrease in susceptibility. Furthermore, the later increase in susceptibility from soaking started at 4 p.m. to 8 p.m. was about 24 hours later than the increase due to immersions started at 4 a.m. to 8 a.m.

There are several ways in which water exerts a deleterious effect on the infection process (5). Superficially the water treatments, which result in increased susceptibility, appear somewhat similar to those which result in decreased susceptibility. At present experiments with different water effects in virus inoculations cannot be adequately reconciled with one another, nor can water effects be adequately reconciled with our knowledge of darkness effects, diurnal effects, and carbohydrate effects.

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THE EFFECT OF HUMIDITY AND ATMOSPHERIC PRESSURE ON VIRUS INFECTION OF LOCAL LESION HOSTS[1]

James D. Panzer[2]

Summary

Nicotiana glutinosa and Phaseolus vulgaris plants inoculated with tobacco mosaic virus and alfalfa mosaic virus, respectively, showed reduced local lesion infection under a high humidity post-inoculation environment. Less infection also occurred at low humidities with bean plants inoculated with alfalfa mosaic virus. When bean and tobacco plants were submerged in water, little infection occurred on the bean plants and infection was absent on the tobacco plants. Plants exposed to various atmospheric pressures after inoculation showed no statistically significant differences in infection.

INTRODUCTION

Humidity as a part of the infection environment of a plant host has been considered and investigated extensively in many of the fungus and bacterial diseases of plants. A comprehensive review of the literature may be found in the work of Delp (1).

However, little work has been done on the effects of humidity on virus infection of local lesion hosts or virus infections in general.

Yarwood (5) discusses the deleterious action of water in plant virus inoculations but limits his investigation to the action of wet and dry inoculation techniques or to the action of a post- or pre-inoculation wetting of the plant. He also gives evidence that with use of aqueous inoculums quick drying of plants resulted in more local lesions than did slow drying. Wet inoculum resulted in less infection than dry, and wilted plants showed more infection than nonwilted.

Inactivation of viruses or virus infection by high mechanical pressures has been demonstrated by various workers. It was established that the tobacco mosaic virus (TMV) became denatured at 7,500 K/sq. cm pressure (3) or 130,000 lbs./sq. in. (2). These investigators used cylinder presses in their experiments. Yarwood (6) found that mechanical pressure before inoculation increased the susceptibility of beans to tobacco necrosis virus and tobacco ringspot virus and of Nicotiana glutinosa to TMV. Pinto beans, resistant to alfalfa mosaic virus (AMV) infection because of age, became susceptible upon exposure to mechanical pressure. No work could be found that related atmospheric pressures to plant virus infection.

MATERIALS AND METHODS

A relative humidity series was established using phosphoric acid-water mixtures of 10, 40, 60, 70, 80, and 85 percent phosphoric acid for relative humidities of 10, 40, 60, 70, 80, and 85 percent, respectively. The solutions were contained in standard desiccator jars, which served as humidity chambers.

Ten days after seeding, common garden bean plants (Phaseolus vulgaris) of the Bountiful and Yelloweye varieties were severed at the crown after leaf inoculation. Their stems were placed in flasks of water in the humidity chambers, where the plants were kept for 36 hours. The same procedure was followed with N. glutinosa plants, except that excised leaves were used.

Standard home pressure cookers were used for atmospheric pressure chambers. A compressed air line was attached to the stem outlet on the top of the pressure cooker. Pressures of 0 to 20 pounds per square inch were possible. Inoculated bean and tobacco plants were placed within these chambers for 36 hours under controlled pressure, and infection allowed to develop.

Plants were inoculated with AMV or TMV by the carborundum-phosphate-rub method (4).

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[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station as Journal Series No. 445.

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Before the inoculated leaves were placed in the humidity chambers, they were allowed to dry to prevent differential drying of the inoculum and inhibition of infection as reported by Yarwood (5).

RESULTS

Humidity Effects

The effect of humidity in the post-inoculation environment on infection by AMV of beans and TMV of tobacco is shown in Figure 1. It is apparent that the high and low humidities result in decreased infection by AMV on bean varieties and that high humidity results in decreased infection of TMV on tobacco.

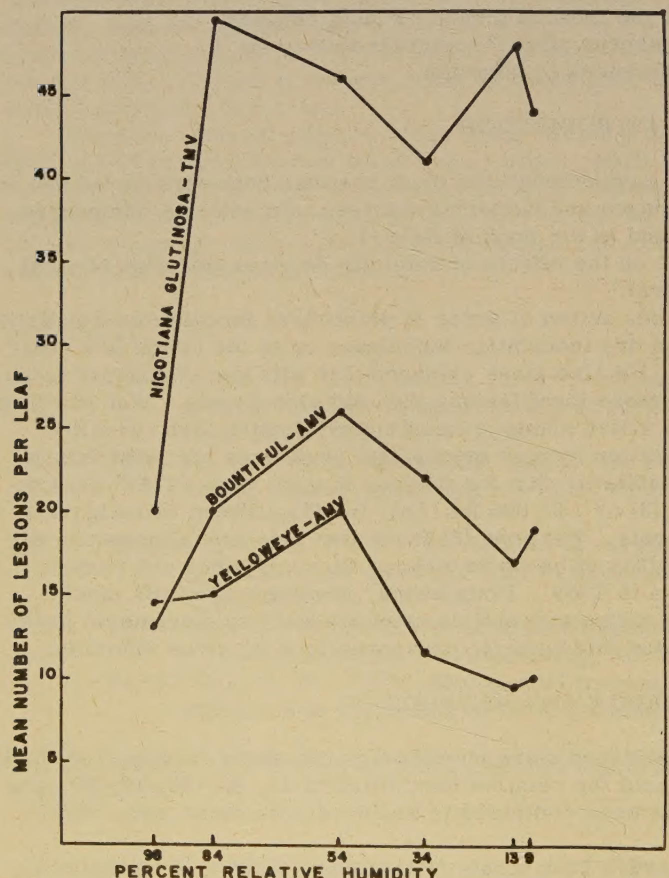

FIGURE 1. The effect of various relative humidities in the post-inoculation environment of virus inoculated host plants. AMV -- alfalfa mosaic virus; TMV -- tobacco mosaic virus.

Table 1. Effect of submergence in water on infection of bean and tobacco plants inoculated with alfalfa mosaic virus and tobacco mosaic virus, respectively.

Host	Mean number of lesions per leaf of: Submerged	Control (not submerged)
Bountiful bean	7[a]	29
Yellow-eye bean	13	37
Nicotiana glutinosa tobacco	0	7

[a]Average of five experiments. 18 leaves per treatment per experiment. All differences between treatment and control significant at the 1% level.

The humidity effect was most pronounced on young bean plants, but did not occur regularly if bean plants of an advanced age (trifoliate leaves developing) were used. Age of plant seemed to have no effect with tobacco.

Lesions that developed on the bean plants under high humidities were irregular and large, while those that developed under lower humidities were more discrete and small (pinpoint). The effect on lesion type was more striking with TMV infection of tobacco (Fig. 2, B).

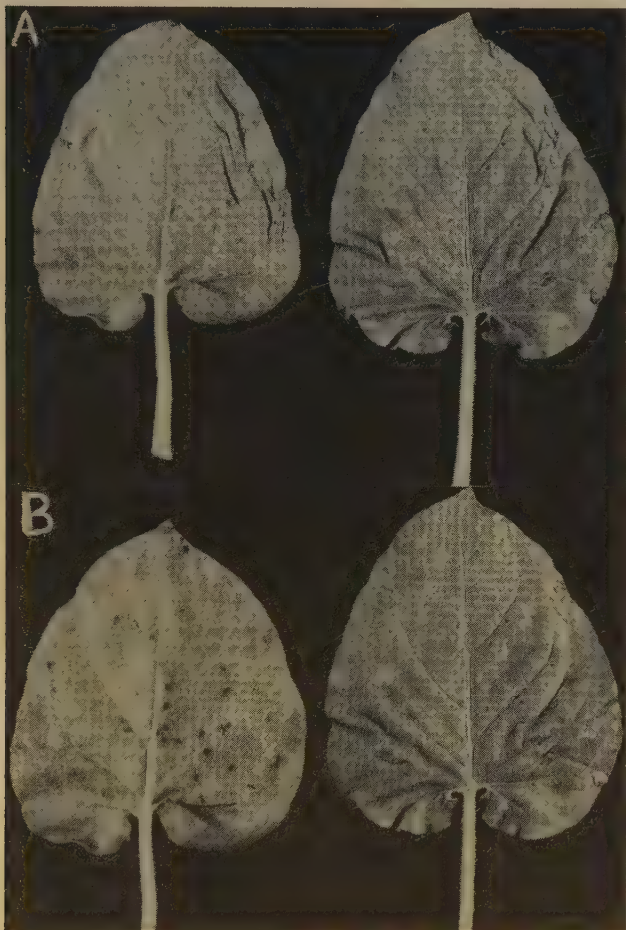

FIGURE 2. Effect of high relative humidity on lesion type of Nicotiana glutinosa plants inoculated with the tobacco mosaic virus. B -- Left leaf exposed to 98 percent relative humidity showing indistinct green blotch lesions; Right leaf (control) exposed to room humidity (35 percent) showing typical local lesions. A -- Same leaves 4 hours after exposure to room humidity.

Fig. 2, B shows infected tobacco leaves immediately after removal from high humidity conditions. The lesions were indistinct blotches of green, and the typical necrotic lesions of TMV infection were limited to the check; however, 4 hours later, after exposure to a relatively dry environment, the indistinct blotch lesions had developed into typical necrotic local lesions (Fig. 2, A).

When bean and tobacco plants were submerged in water after inoculation, limited infection occurred (Table 1).

Pressure Effects

No effect on infection with either AMV or TMV was noted upon exposure of inoculated plants to atmospheric pressures as high as 20 pounds per square inch.

DISCUSSION AND CONCLUSIONS

It has been demonstrated that relative humidity has an effect upon virus infection of local lesion host plants. High humidity in the post-inoculation environment appears detrimental to infection. Where tobacco plants were submerged in water, no TMV infection was found.

The limited infection with bean varieties at low humidities, but no apparent effect with tobacco, may be due to a host effect or to the nature of the virus, since TMV is a relatively more stable virus than AMV. Another environmental factor resulting in marked effects upon AMV infection but not upon TMV infection has been observed by the author (4).

The large, irregular lesions on the bean plants and the indistinct green blotch lesions on the tobacco under high humidity conditions approach a systemic type of infection when contrasted with the typical necrotic local (hypersusceptible) reactions found at the lower humidities. This might indicate that lesion type could be related to the number of secondary infection loci, the cells surrounding initial infection loci becoming less susceptible.

At the low extreme in humidity, the slight rise in number of AMV lesions on beans may be explained from the data of Yarwood (5), who found that wilted plants exhibited more lesions than turgid plants. Since a few of the plants became wilted under the very low humidities, the author feels that Yarwood's findings explain the slight increase in number of lesions.

Since the differences in atmospheric pressure had no effect upon virus infections, the mechanical pressure effects noted by others (2, 3, 6) may have resulted from physical denaturation of the virus protein, or the host tissue may have been injured. Atmospheric pressure is equal upon all sides of the tissue, and thus differential injurious effects should not result and the effect of pressure per se may be tested.

When quantitative studies are made with virus infection, it is suggested that the humidity in the post-inoculation environment of local lesion test plants be as stable as possible, to assure more uniform assay plants.

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BARLEY YELLOW DWARF VIRUS ON CEREALS IN ONTARIO[1]

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Summary

Symptoms of barley yellow dwarf have been observed on cereals in southeastern Ontario for several years. In 1958 about 15 percent of the oats and barley and a smaller percentage of the wheat grown in the Ottawa Valley developed symptoms by mid-July. In oats the proportion of diseased plants was higher when the plants were widely spaced than when closely planted in rows, when sown late than when sown early, and when grown near unsprayed apple and *Prunus padus* trees. Three species of aphids found on diseased plants in the field proved infective with BYDV when fed on Clintland oats, as follows: *Rhopalosiphum padi* from wheat, oats and barley, *R. maidis* from spring barley, and *Macrosiphum avenae* (=*granarium*) from both winter and spring varieties of barley and wheat. Non-viruliferous *R. padi* was generally the most efficient vector for isolating the virus from naturally diseased plants, but it failed to transmit virus from several collections of winter and spring barley and winter wheat from which BYDV was transmitted with *M. avenae*. *R. maidis* transmitted to a low proportion of the test plants from naturally diseased barley. The destructiveness of the virus was demonstrated in a field experiment in which viruliferous and non-viruliferous *R. padi* were caged on oats and barley for 1 week at each of three stages of development. When aphids that carried BYDV isolated from timothy fed on Clintland oats, Garry oats, and Montcalm barley in the 3- to 4-leaf stage, the plants of these varieties became chlorotic and severely stunted and the yields were reduced by 75.3, 77.6, and 53.5 percent, respectively. A similar infestation 2 weeks later reduced the yields by 56.1, 59.7, and 23 percent, respectively. The last infestation, when the plants were in the jointing stage, caused no measurable yield loss. York barley was affected only mildly even by the earliest infestation. Non-viruliferous aphids caused no increase in disease or reduction in yield of any of the varieties.

INTRODUCTION

For several years, chlorotic and often stunted cereal crop plants have been observed in experimental plots and farmers' fields in southeastern Ontario. The chlorosis usually developed downward from the leaf tips, but sometimes chlorotic blotches developed. Wheat and barley leaves turned various shades of yellow. Oat leaves often initially showed water-soaked areas, then turned dull yellow in color, but varying degrees of reddening were sometimes associated with the chlorosis. These symptoms are similar to the symptoms usually associated with the barley yellow dwarf virus (BYDV), which has a wide range of graminaceous hosts and has been reported from many areas of the United States, the Canadian prairies, and most countries in Northern Europe (1, 2, 3, 6, 7, 8, 9, 11, 12).

The apparent crop damage associated with the occasional high incidence of the disease in

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southeastern Ontario indicated a practical need for a study of the cause, sources, and potential destructiveness of the disease in the area. Since many factors may induce pigment disorders in cereals (5), it appeared necessary to do aphid transmission studies to determine if BYDV was the cause of any of the disease symptoms observed.

METHODS OF TESTING FOR BYDV

In the fall of 1957, transmission experiments were begun with Rhopalosiphum padi (L.) found on naturally diseased oats. Non-infective cultures of aphids were developed from nymphs produced by females confined on moist filter paper. The first sources of virus tested were chlorotic stunted oats and timothy. Leaves from the diseased plants were placed in a beaker with moist sand, the non-viruliferous aphids were placed on them, and the beaker was covered with filter paper held in place with a rubber band and moistened occasionally. After a 2-day aquisition feed up to five aphids per plant were placed on cereal plants in the 1- to 2-leaf stage: 2 to 3 days later the aphids were killed by fumigation. Several varieties of cereals were compared as test plants, including the barley varieties Atlas, Brant, Blackhulless, Club Mariout, Montcalm, Parkland, Tennessee Winter, Vantage, and York; the oat varieties Clintland, Garry, Rodney, and Winter Turf; and the wheat variety Cornell. Varying numbers of plants of the wheat and barley varieties developed mild to moderate symptoms. All the oat varieties proved highly susceptible. Clintland appeared to be most susceptible and since it was readily available it was used as the regular test variety. At greenhouse temperatures near 20° C symptoms usually began to appear on Clintland plants about 10 days after infective aphids had fed on them. The earliest symptoms on Clintland were usually water-soaked blotches that later turned chlorotic. Infected plants were stunted to varying degrees, depending on the isolate of the virus. Some isolates arrested the development of new leaves.

VARIATION IN THE DISTRIBUTION OF BYDV IN THE FIELD

At weekly intervals during the summer of 1958 estimates were made of the percentage of plants with symptoms attributable to BYDV in various cereal plots and five specially planted observation plots. Each of the latter consisted of about 100 plants of each of Clintland oats and York barley planted at various locations on the Central Experimental Farm. One of the observation plots was located adjacent to plantings of various trees and shrubs, including apple and cherry trees. Three others were surrounded by various plots of oats and barley in large fields, and another was surrounded by plots of winter wheat.

Yellow dwarf symptoms were first noticed on June 2 on winter wheat plants which probably became infected the previous fall. On June 20 one infected oat plant was observed in one of the observation plots. On June 27 traces of infection were observed in grains in the vicinity of all the observation plots, but chlorotic leaves characteristic of yellow dwarf were found in only two of the plots. The highest incidence of symptoms occurred adjacent to the plantings of trees and shrubs including Malus spp. and Prunus padus L. (European bird cherry) where, on June 27, 8 percent of the Clintland Oats and 13 percent of the York barley in the observation plots had chlorotic leaves. This contrasts with 0 to 2 percent of the plants with symptoms in the plots at other locations. By July 18, 20 percent of the oats and 40 percent of the barley plants were diseased in the plots near the fruit trees. In the other plots only 7 to 14 percent of the Clintland, and 2 to 18 percent of the York plants had chlorotic leaves. It appears likely that apple, which is an overwintering host of Rhopalosiphum fitchii (Sand.), and cherry, which is an overwintering host of R. padi, may have favored early infection of the cereals in the vicinity. By July 25, 21 to 35 percent of the oats and barley in all the observation plots had symptoms, with little variation between locations.

A high incidence of the disease in one of the oat nurseries appeared to be correlated with the wide spacing of plants. On July 18 between 25 and 50 percent of the plants were diseased in plots where the plants were spaced 1 to 2 feet apart in the rows. In contrast, only 10 percent of the plants were diseased in rows where the plants were 2 to 3 inches apart.

Thirty-two varieties and strains of oats grown in drill width strips 6 x 100 feet were rated for percentage of plants showing symptoms indicative of yellow dwarf infection. The strips were separated by a clipped area 10 feet wide. No artificial inoculation was made. Infection ratings made July 25 ranged from a trace to 35 percent with most varieties being in the 15 to 20 percent range.

According to a survey of 17 fields made on July 17 and 18, it was estimated that about 15 percent of the oats and barley in the Ottawa area was affected by the barley yellow dwarf virus.

A spotty distribution of diseased plants was apparent in the larger plots and fields of oats. Frequently areas several feet in diameter were found in which almost all plants were diseased and some were severely stunted, but in the remainder of the field the diseased plants were scattered and only slightly stunted. It appears that the patches of diseased plants resulted from primary infestations of viruliferous aphids that came into the field early in the growing season.

APHIDS ASSOCIATED WITH BYDV IN THE FIELD

From late May on throughout the summer of 1958, Rhopalosiphum padi occurred in light to moderate numbers on oats, barley, and wheat. These aphids appeared to be primarily responsible for yellow dwarf infections in oats. BYDV was transmitted to test plants in the greenhouse by R. padi, found not only on naturally diseased oats, but also on barley and wheat in the field. The English grain aphid Macrosiphum avenae (Fab.) (= granarium (Kirby)) was common but not abundant on barley and wheat, and aphids of this species collected on naturally diseased wheat and barley proved infective when tested on Clintland oats. The corn aphid, Rhopalosiphum maidis (Fitch), was abundant on barley during late June and July but caused little apparent feeding injury. Two collections of this aphid proved infective when tested on Clintland oats. Species of Sipha (Pass) and Rungsia (Mimeur) found on diseased barley and wheat did not prove infective.

Non-viruliferous R. padi was also used to verify the presence of BYDV in leaves of diseased spring barley and oats collected in experimental plots. The first attempt to isolate BYDV from several samples of winter barley and winter wheat found in experimental variety nurseries failed when R. padi was used as the vector. The tests were repeated with fresh samples from the same plots, using non-viruliferous M. avenae as well as R. padi as vectors. Again, R. padi failed to induce symptoms but M. avenae that had fed on either winter barley or winter wheat induced mild chlorotic symptoms, on Clintland oat test plants. These results indicate that the winter barley and winter wheat may have been infected with a strain of virus readily transmitted by M. avenae but not by R. padi. Rochow (10) has reported vector specific strains of BYDV in New York. Perhaps a similar situation occurs in the Ottawa area.

Isolates of BYDV varied considerably in the degree of stunting that they caused on Clintland oats. However, an isolate transmitted by R. padi from a stunted chlorotic timothy plant caused more severe stunting in all tests in which it was used than any of the isolates obtained from barley, oats or wheat (Figure 1).

FIGURE 1. Clintland oats following artificial infestation with Rhopalosiphum padi. Left, non-viruliferous aphids; center, aphids carrying BYDV isolated from oats; right, aphids carrying BYDV isolated from timothy.

DATE OF SEEDING IN RELATION TO INFECTION OF OATS

A duplicate test of the four oat varieties Abegweit, Fundy, Garry, and Victory was seeded in rod rows on May 1, May 15, May 29, and June 11, 1958. The diseased plants were counted periodically during the summer. On July 11 1.3 percent of the plants in the May 1 seeding and 4 percent in the May 15 seeding showed chlorotic leaf symptoms indicative of BYDV infection. None of the plants in the two later-sown plots had yet developed symptoms of disease. On July 22, the percentages of diseased plants in the plots, in order of date of seeding, were 4.5, 6.6, 5.4, and 9.1 respectively. By August 18 the plants in the plots sown May 1 were too near maturity to permit a reliable distinction of plants with symptoms, but in the later-sown plots percentages of diseased plants recognized were 15, 16.2 and 40, the highest percentage being in the plots sown latest. Allowing 2 to 3 weeks after infection for symptoms to become apparent, it seems that the greatest spread of virus occurred during mid-July, at which time the oats sown June 11 were still in an immature vegetative stage. Differences among the four varieties in percentages of plants that became diseased did not appear to be significant.

RELATION OF TIME OF INFECTION TO THE EFFECTS OF BARLEY YELLOW DWARF VIRUS ON OATS AND BARLEY IN THE FIELD

The effects of viruliferous and non-viruliferous Rhopalosiphum padi when fed on oats and barley at three stages of development were compared in a field experiment in 1958. Non-viruliferous aphids were reared on wheat and crested wheat grass in the greenhouse. Aphids carrying BYDV isolated from timothy were multiplied on Clintland oats. Plots for the tests were sown May 22. Each plot consisted of two rows 9 inches apart and 6 feet long, of each of York barley, Montcalm barley, Clintland oats, and Garry oats. There were 13 plots in each of four replicates. On each of three dates, June 6, June 20, and July 4, when the plants were in the 3- to 4-leaf, stooling, and jointing stages respectively, non-viruliferous aphids were put on one plot in each replicate, and viruliferous aphids on another. None of the alternate plots were infested, and they served as checks. To control the spread of the aphids, two cages 1 meter square and 1 meter high, covered with 32-mesh/inch lumite saran screen, were used to cover the area to be infected in each plot. About 200 aphids were applied to each meter-length row. Uniform application of aphids was achieved by first distributing the insects for one row of plants along a transparent plastic trough the length of the row, then inverting the trough over the row and tapping until all aphids fell onto the plants. After 1 week the caged plants were sprayed with malathion to kill the aphids, but the cages were not removed until the following week.

The plants in the plots experimentally infested with viruliferous aphids on June 6 and June 20 began to show chlorotic leaf symptoms during the third week after infestation. By July 14, when the plants were fully headed, these areas in the plots stood out in sharp contrast because of the high percentage of chlorotic and severely stunted plants occurring within the area as compared with the low incidence of diseased plants in the remainder of the plots (Table1). Clintland and Garry Oats proved highly susceptible to infection, and both varieties were severely stunted, but most severely by the earlier infestation. Montcalm barley was not quite as severely affected as the oats when infested on June 20. York barley was only slightly affected by the viruliferous aphids that were put on the plants. The plots on which the non-viruliferous aphids had fed contained no more diseased plants than the alternate check plots throughout the planting which were not experimentally infested with aphids. However, natural infestations with aphids had occurred in all, and the percentage of plants of the various varieties that developed chlorotic leaves was 10 for York barley and about 4 for Montcalm barley and the two oat varieties. None of the varieties in plots infested on July 4 developed symptoms that could be attributed to the experimental treatments. By the time symptoms should have become apparent, other diseases and the effects of lodging and maturity obscured any symptoms that could have been attributed to barley yellow dwarf virus.

At maturity the plots were harvested despite severe lodging. Plots which were most severely affected by BYDV were least affected by lodging. The yields in grams from the two meter-length rows of each variety for each treatment are shown in Table 2 and are the averages from the four replicates. The yields of adjacent non-infested rows are also shown. The yields in plots on which non-viruliferous aphids were caged on June 6 and June 20 are not significantly different from those in adjacent plots that were not experimentally infested. In the plots infested with viruliferous aphids on June 6, the yields of Clintland, Garry, and Montcalm were

Table 1. Relation of barley yellow dwarf virus to chlorosis and stunting of oats and barley in field plots experimentally infested with Rhopalosiphum padi.

Aphids	Date applied	Variety	Percent of plants chlorotic[a]	Percent stunting caused by introduced aphids
Viruliferous	June 6	Clintland oats	100	72
		Garry oats	100	72
		York barley	23	20
		Montcalm barley	100	60
Non-viruliferous	June 6	Clintland oats	6	0
		Garry oats	5	0
		York barley	10	0
		Montcalm barley	3	0
Viruliferous	June 20	Clintland oats	100	46
		Garry oats	100	36
		York barley	10	13
		Montcalm barley	72	23
Non-viruliferous	June 20	Clintland oats	6	0
		Garry oats	5	0
		York barley	10	0
		Montcalm barley	3	0

[a] Estimations of chlorosis and measurements of stunting made on July 14 are the average of 4 replicates each containing about 60 plants of each variety.

Table 2. Relation of barley yellow dwarf virus to the yields of oats and barley in field plots experimentally infested with Rhopalosiphum padi.

Treatment	Grain yields in grams per 2 meters of row: Oats: Clintland	Oats: Garry	Barley: York	Barley: Montcalm
Viruliferous aphids applied June 6	26.0	31.5	175.0	33.5
Average of 2 adjacent check plots	95.0	121.5	136.3	79.9
Non-viruliferous aphids applied June 6	105.5	140.5	140.3	72.0
Average of 2 adjacent check plots	96.7	139.2	106.1	88.0
Viruliferous aphids applied June 20	46.2	72.2	103.2	74.7
Average of 2 adjacent check plots	79.7	133.2	128.6	86.9
Non-viruliferous aphids applied June 20	105.2	179.0	133.2	97.0
Average 2 adjacent check plots	99.5	132.2	141.9	90.6
Viruliferous aphids applied July 4	86.2	98.0	116.2	81.0
Average 2 adjacent check plots	96.2	123.2	137.9	72.1
Non-viruliferous aphids applied July 4	63.0	118.0	84.0	64.0
Average 2 adjacent check plots	80.2	132.2	111.5	67.0

75.3, 77.6, and 53.5 percent lower, respectively, than those of the same varieties in plots infested with non-viruliferous aphids. In the plots infested June 20, the yields of the same varieties were reduced 56.1, 59.7, and 23 percent. The yields of York barley were not reduced significantly by the viruliferous aphids even in the plots infested earliest. None of the oat or barley varieties appeared to be significantly affected by the aphids placed on them on July 4, but the plants caged at this date appeared slightly more affected by lodging which contributed to variations in yield.

A striking, although not surprising, feature of this experiment was the severe effects of the early infestation in contrast with the insignificant effects of the later infestation made when the plants were in the jointing stage. These results parallel the results of the experiment by Endo (4). A more surprising result is that by June 20 some of the plants were already showing BYD symptoms from natural infection, yet there was no apparent increase of virus-infected plants in the plot as a result of infestation with non-viruliferous _R. padi_, which might be expected to acquire virus from the few diseased plants and transmit it to others during the week. The lack of spread in the plots may merely have been because the aphids did not move appreciably from plant to plant, or perhaps the virus occurring naturally in the plots was not readily transmissible by _R. padi_. Samples of diseased barley from the plots were tested in the greenhouse for transmission by _R. padi_ and _M. avenae_. Only the latter transmitted virus from the samples, thus indicating a vector specificity similar to that reported by Rochow (10).

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CANADA DEPARTMENT OF AGRICULTURE

EPIDEMIOLOGY OF STEM RUST: II. (RELATION OF QUANTITY OF INOCULUM AND GROWTH STAGE OF WHEAT AND RYE AT INFECTION TO YIELD REDUCTION BY STEM RUST)

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Summary

In inoculated wheat plots almost complete loss of yield (42 bushels per acre in check versus 0.9 bushels) was obtained in those plots in which rust severity reached 1 percent by the early boot stage. Yield reductions were progressively less when this severity was attained at early heading, late heading, milk, and grain development respectively. While similar relationships between severities attained in relation to host stage were demonstrated in rye plots, yield reductions were not so severe even though there were greater initial levels of infection.

Terminal infection severity represents the culmination of the epiphytotic that began when the inoculum was first applied. The amount of initial infection and the stage of plant growth at which it is induced are major factors in determining the extent of rust development. The relative influences of these two factors in reducing yield were the subjects of this study.

MATERIALS AND METHODS

Plot Preparation and Seeding

Thorne wheat was sown October 8, 1953 on the eastern 4.8 acres of the 9.5 acre field at Fort Detrick. The soil was a Duffield silt loam. Seed was drilled in rows 7 inches apart, at 96 pounds per acre. The plants emerged on October 20. Abruzzi rye was drilled on the western half of the field in rows 7 inches apart, at 99 pounds per acre; plants emerged October 13. Fertilizer (5-10-5) was drilled along with the seed, at 400 pounds for the wheat and 300 pounds for the rye.

Weather Instruments

Weather records, including temperature, relative humidity, and duration of dew, were maintained from the time of the first inoculation throughout the experiment. Temperature and relative humidity were measured by hygrothermographs located at ground level in the wheat plots and about 4 feet above the ground in a weather shelter. Duration of dew was recorded by a Taylor dew meter[3] located in the open near the weather shelter. The recording plate of the meter was 10 inches above the ground. Rainfall was measured in a standard rain gauge. Wind direction and velocity were measured by a Bendix-Friez aerovane located between the wheat and rye fields about 15 feet above ground level.

Inoculation

Plants were inoculated with 0, 0.1, 1.0, 10, and 100 grams of rust spores per acre. Plots were arranged in a 5 X 5 Latin square in both wheat and rye fields. Rye was heading and the wheat was jointing when inoculated on April 20 and 22. Inoculum for wheat (*Puccinia graminis tritici*) and rye (*P. graminis secalis*) germinated 84 and 87 percent respectively.

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[1]Quality Maintenance and Improvement Section, Agricultural Marketing Service, United States Department of Agriculture, Beltsville, Maryland.

[2]United States Army Chemical Center, Fort Detrick, Frederick, Maryland.

[3]Taylor, Carlton F. 1956. A device for recording the duration of dew deposits. Plant Disease Reptr. 40: 1025-1028.

Inoculum for each plot was weighed and thoroughly mixed[4] in quart jars with 150 grams of talc. Dilution of spores in talc facilitated uniform deposition. Inoculation was accomplished during inversion at 1800 hours with Admiral No. 766 dusters manufactured by the H. D. Hudson Manufacturing Co. The 20 X 20 foot plots in 5 X 5 Latin squares (one in the wheat and one in the rye) were separated from neighboring plots by uninoculated strips 20 feet wide to provide a buffer zone for reducing interplot spread of spores. Ten-foot wide strips were also left between the plots and the edge of the field. The resulting test area was a square 200 feet on each side. The buffer zone between the plots failed to prevent rust interplot spread, and uninoculated plots in the test area became severely infected after the second rust development cycle.

Uninoculated wheat plots farther from the test area but in the same field were dusted several times with sulfur in an attempt to provide rust-free control plots. This attempt failed, however, and it was necessary to use samples from another field as rust-free checks. These plots were located about 1/4 of a mile upwind from the test area and were seeded with Thorne wheat on October 8 in the same manner as the test field. Only a trace of rust was found in this field at harvest. Since the agronomic conditions in this field were similar to those in the test area and since the rust infection was negligible, it was considered that grain harvested from it provided a valid basis for comparisons. Yields from uninfected rye plants in another part of the test area served as controls.

In addition to the recording instruments used at the south edge of the field, dew meters were placed in each plot inoculated at the 100-gram rate on the night of inoculation only. On other nights a dew record was secured from a dew meter in the open near the instrument shelter, with the record plate 10 inches above the soil. Yield data were obtained by harvesting grain from all plants in a 10-foot square in the center of each plot. The plants were cut by hand and threshed in the field with the blower of the thresher reduced in speed to minimize loss of grain. Grain was separated from the chaff with a seed cleaner before being weighed to determine yield.

Rust Development Measurements

Development of infection in the plots was observed either by counting the individual pustules on the culms or by estimating the severity of the infection according to the modified Cobb scale. The two initial series of plot readings were made by examining groups of 20 successive culms from 25 locations in a 5-by-5 foot pattern within each plot. This relatively large number of observations was used to study the uniformity of initial infection and the variability of early rust spread within the plot. This sampling method required 1/2 man-day per plot.

By examination of the data from this intensive sampling procedure it was determined that satisfactory estimates of rust intensity could be obtained by counting pustules on 10 culms in each of 5 locations -- one at plot center and one 4 feet in toward the center from each corner of the plot.

When total numbers and coalescence of pustules made accurate counts by this method impracticable, disease severity was estimated in percent. The relationship between the two systems at the point where one is abandoned in favor of the other is indicated in Table 1.

Table 1. Comparison of pustule counts with severity estimates.

	Trace severity	1 percent severity
Number pustules per 20 culms	82	200
	79	186
	33	288
	147	83
	57	224
	91	302
Mean	81	214
Pustule number/severity ratio	4	10.5

[4]A small Twin-shell dry blender (Patterson-Kelley Co., Inc., East Stroudsburg, Pa.) was used for this purpose.

These data illustrate to some degree the variability of numbers of pustules in relation to "trace" and "1 percent severity" estimates by the workers. On the basis of this and other data, 10 pustules per culm was considered equivalent to 1 percent severity.

RESULTS

Effect of Initial Dose of Inoculum

The infections that resulted from the rye stem rust inoculations made April 20 and the wheat stem rust inoculations made April 22 were satisfactory. In wheat the instensity ranged from 5 pustules per 1000 culms to 42 pustules per culm in the plots inoculated at low and high rates. The number of infections in the rye plots was substantially higher and ranged from 100 per 1000 culms to 218 per culm. The rates of inoculum applied and the levels of infection observed 14 days later are shown in rounded numbers in Table 2. Increase in infection with increased dose was somewhat greater in wheat than it was in rye. The 10-fold increments in amount of inoculum applied increased infection in wheat 5, 10, and 42 times and in rye 8, 10, and 13.5 times. This graded series of initial infections provided an excellent starting point for measuring relative rates of rust development with initial infection being the only variable.

Table 2. Infection resulting 14 days after initial inoculation.

Rate of inoculation in grams per acre	Pustules per 1000 culms: Wheat	Pustules per 1000 culms: Rye
Check	5	100
0.1	20	200
1.0	100	1600
10.0	1000	16000
100.0	42000	218000

Effect of Temperature

The general rate of development of the wheat stem rust, as shown in Table 3, was relatively slow from May 10, when the first infection was observed, to May 28. After May 28 a very rapid increase occurred in all plots. The mean temperature for the first 12 days after inoculation was 61° F. This period was followed by an interval of 20 days during which the mean temperature exceeded 60° on only 1 day.

Table 3. Stem rust development in wheat plots.

Observation date	Development stage of host	Infection from indicated inoculation rate (grams/acre): 0	0.1	1	10	100
May 10	Boot	0.005[a]	0.2	0.1	1.0	4%
20	Late boot-head	0.1	0.1	0.7	8.8	5%
28	Head	0.4	0.4	4.3	1%	8%
June 2	Flowering	5.3	5.3	2%	15%	56%
8	Milk	14%	14%	35%	61%	87%
14	Dough	60%[b]	64%	70%	82%	95%

[a]Average number of pustules per culm (5 replicates), or percent severity.
[b]Plots in same test area originally designated controls.

The temperature began to rise on May 24 and fell below 60° F only once in the following 35 days. The three stages of epidemic development are indicated in Table 4, with the mean temperatures during each period. Effect of the lag is particularly evident in plots inoculated at 100 grams per acre. The tremendous increase in severity noted on June 2 reflected an accumulation of infections that had been initiated over a period of time. The development and eruption of these pustules was delayed by low temperature. Since the wheat was heading when the

Table 4. Temperature means during development of wheat stem rust epiphytotic.

Stage of epidemic development	Mean temperature °F	Dates (inclusive)
Incubation -- First cycle	61.4	April 22-May 3
Incubation -- Second cycle	48.7	May 4-May 13
	55.3	May 14-May 23
Period of rapid outbreak	62.8	May 24-May 28
	67.4	May 29-June 2
	63.6	June 3-June 7
	71.2	June 8-June 12
	67.8	June 13-June 17

Table 5. Stem rust development in rye plots.

Observation date	Development stage of host	Infection from indicated inoculation rate (grams/acre)				
		0	0.1	1	10	100
May 7	Flowering	0.1[a]	0.2	1.6	2%	21%
18	Flowering	0.3	3.1	1%	7%	30%
27	Milk	5.2	10.8	5%	16%	40%
June 1	Soft dough	1%	2%	10%	28%	48%
8	Dough	17%	17%	29%	34%	47%

[a]Average reading from 5 replicates expressed as number of pustules per culm or percent severity.

upward swing in temperatures began, ample time was provided for the rust to increase before the plants ripened.

Rye matures more rapidly than does wheat, and by the time the low temperature period ended the plants were flowering. Shortly after flowering, rye characteristically becomes much less succulent and enters a fairly long ripening period. This earlier flowering and loss of succulence considerably shortened the period during which both suitable environment and susceptible host tissue were present, and consequently reduced the terminal severity of the rye rust. This is apparent when the data in Tables 3 and 5 are compared.

Spread of Rust from the Inoculated Areas

Little spread into the borders was noted until after the second cycle of rust had developed. There was light initial infection in the original check plots from drift during inoculation of plots at the higher rates. Following the second cycle, infection became general through the experimental area. Interplot contamination was overshadowed in the three higher rate plots because of the heavy infection resulting from inoculation. The parallel development of rust in the check and 0.1-gram per acre wheat plots indicated a uniform distribution over the entire area of inoculum that was at least equal to that being produced in the 0.1-gram per acre plots, and may have exceeded it. The terminal severities attained both in the check and in the plots inoculated at 0.1 gram per acre reflected again the magnitude of the interplot spread. Spread from the inoculated area into the remainder of the field was very appreciable. By June 3 plants five rows distant from the inoculated plots had counts as high as 27 pustules per culm; on the same date, however, the count at row 18 was less than one pustule per culm.

By June 9 severity ranged from 45 percent in the adjacent rows to trace severity at row 30. By June 15 it had increased to 85 and 45 percent respectively. Repeated applications of sulfur to plots at the far edge of the field succeeded only in slightly delaying the rust development.

Reduction in Grain Yield

The relation between severity of stem rust and reduction in yield was clear cut. Time of

FIGURE 1. Grain from rust free plants (left) and from heavily rusted plants (right).

attainment of 1 percent severity in relation to the stage of growth of the plants and to ensuing damage is revealed by the data summarized in Table 6, which shows the average grain yield in each plot along with date and stage of crop growth at which infection reached 1 percent severity.

Table 6. Relation between yield of grain and timing of rust infection.

Rate of inoculation (grams)	Wheat: Date of 1% severity	Wheat: Host stage at 1% severity	Wheat: Yield bu/A[a]	Rye: Date of 1% severity	Rye: Host stage at 1% severity	Rye: Yield bu/A[b]
Check	July 2[c]	Ripe	43.1	Never attained	Ripe	39.8
0.0	June 6-7	Flower-milk	11.6	May 29-30	Milk-soft dough	24.4
0.1	June 6-7	Flower-milk	8.6	May 29-30	Milk-soft dough	24.6
1.0	May 29-30	Head-flower	7.5	May 19-20	Milk-soft dough	23.9
10.0	May 23-24	Boot to head	4.1	May 5-6	Early flowering	17.8
100.0	May 9-10	Boot	0.9	May 3-4	Heading	11.6

[a]LSD 5% = 3.2 bu/A; 1% = 4.5 bu/A
[b]LSD 5% = 5.0 bu/A; 1% = 6.8 bu/A
[c]Check from field upwind from inoculated area. Rust never exceeded trace severity.

Reduction in yield was greatest in the plot in which rust developed first. A trace of infection on plants in the early boot stage built up to approximately 55 percent severity at time of flowering. This amount of rust caused a marked reduction in the set of grain, and kernels which did form were small and shrivelled. Grain damage is shown in Figure 1. Inoculum spreading from the higher rate plots affected, to a large extent, the terminal severity of rust and the yield in all plots and samples from surrounding border areas.

Wheat kernel weight and germination of seed from the various plots are given in Table 7. Viability counts were made shortly after harvest and do not indicate any subsequent deterioration in storage.

Table 7. Weight and germinability of grain from rusted wheat plants.

Rate of inoculation	Date at which 1% severity was attained	Weight of 100 kernels (grams)[a]	Germination of seed, percent[b]
Check	July 2	3.66	98
None	June 6-7	1.44	82
0.1	June 6-7	0.98	72
1.0	May 29-30	0.88	60
10.0	May 23-24	1.03	53
100.0	May 9-10	0.42	1
	LSD 1%	1.62	15

[a]Values are means of three counts of 100 kernels per count.
[b]Values are means of two counts of 100 kernels per count. The germination test interval was 6 days at 65° F.

The yield figures for the rye plots (Table 6) reflect the characteristic early loss of succulence in rye plants and the effect of late infection. A trace of infection attained as the berry was beginning to firm up into a grain could be expected to cause little reduction in yield. Little damage was apparent even when the infection reached a trace level during late flowering. A 25 percent reduction in yield resulted when a trace severity was present as the first plants began to flower, and 50 percent loss occurred when 1 percent severity was reached at heading.

The terminal rust severity is plotted against yield of wheat in Figure 2.

DISCUSSION

Winter wheat retains its green color longer in the spring than does rye. This indicates a difference in speed of host maturation which accounts in large measure for the greater destruc-

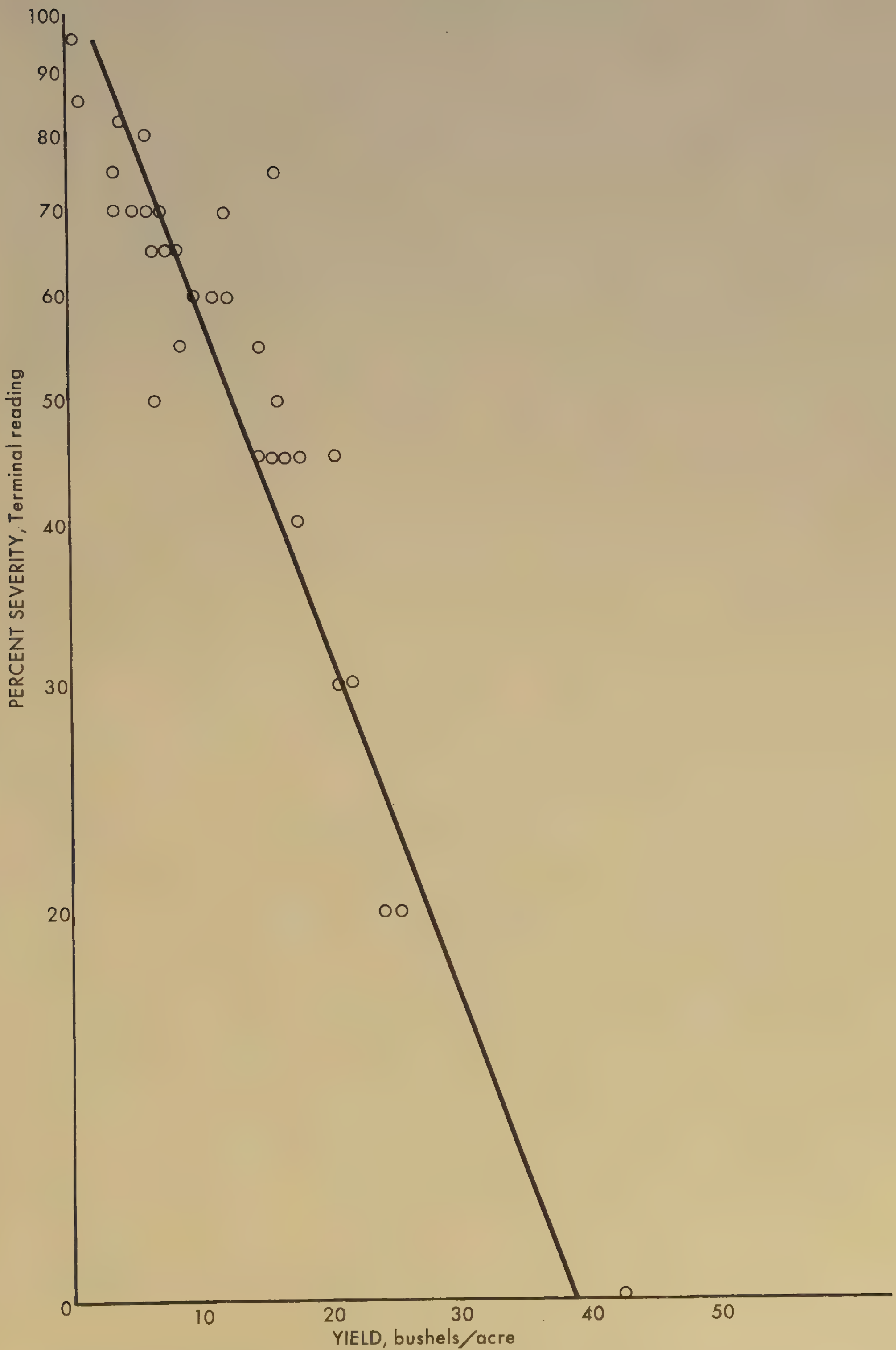

Figure 2. Relation of terminal rust severity to grain yield .

tiveness of stem rust on wheat, even though initial infection took place on both hosts at the same time. Initial infection was higher in the rye plots than in the wheat plots, nevertheless terminal severity on rye did not approach that on wheat. During the cold period of May 4 to 14 rye was less retarded than wheat, and at the same time it was much less affected by the low temperatures than the rust to which it was host.

Results in the wheat plots were impressive, with regard both to the amount of infection and the rate at which it developed. The effects of the lower rates of inoculation are not clearly shown because of the amount of interference by contamination from adjoining plots. While there was a 75 percent reduction in yield of the uninoculated plots, it does not follow that this constitutes a base figure that should be subtracted from the yield reduction in all plots. The interplot contamination did not overshadow the results on plots inoculated at the higher rates. Drift of inoculum from high rate to lower rate plots during inoculation and during the experiment did produce the effect of one less cycle to significant destruction in the lower rate plots. The test demonstrated that a small amount of inoculum arriving early enough to permit an additional cycle of infection can cause more destruction than ten times this amount of inoculum arriving at a later developmental stage of the host. Although it is unlikely that the yield reduction would have amounted to 74 percent in the plots inoculated at the rate of 0.1 gram per acre, reduction would nevertheless have been substantial as severity reached 1 percent before the explosive outbreak of rust obliterated initial distinctions among plots, borders, and uninoculated areas.

FORT DETRICK, FREDERICK, MARYLAND

CORN STALK ROT TRIALS IN PENNSYLVANIA, 1958[1]

C. C. Wernham

Abstract

In a northeastern regional experiment, six inbred lines of corn were compared for stalk rot reaction, using different methods of attack. The classical concept of tissue discoloration and destruction does not yield the same results as the practical concept of "standability" employed by the agronomists. Even though agronomic results vary from station to station, the concept of "standability" appears to be the one best suited for protection of the farmer against stalk rot losses. The variability of reaction in some long-time inbreds suggests that reselection or "sub-lining" might prove to be profitable.

Stalk rot is the most persistent and troublesome disease of corn in Pennsylvania. This statement applies throughout the corn-growing areas of the Northeastern States (Maine, Vermont, New Hampshire, New York, Massachusetts, Rhode Island, Connecticut, Pennsylvania, New Jersey, Delaware, Maryland, West Virginia) and Canada (Ontario, Quebec). These areas are characterized by a wide variance in growing conditions for which a wide array of corn hybrids has been recommended. In Pennsylvania alone hybrids range in maturity from the "200" to the "800" class. Delaware, Maryland and parts of New Jersey use even later hybrids.

With such an array of hybrids and the wide variances in soil type, length of season, rainfall, and temperature, much confusion exists about the performance of corn hybrids with respect to stalk rot. The confusion arises from two origins: 1) the particular cause of stalk rot, and 2) the interpretation of phenomena attributed to stalk rot.

Unlike the corn belt, in the Northeast it is yet to be demonstrated that Diplodia maydis (D. zeae) plays even a minor role as a causal agent of stalk-rot. In the corn disease nursery at the Pennsylvania Agricultural Experiment Station, all maize cultures grown through 1957 have been inoculated with mixtures of Diplodia maydis and Gibberella zeae. Diplodia never persists. It is never found in non-inoculated border rows nor in adjacent fields.

All intensive studies of stalk-rotted plants have revealed only Gibberella components as causal agents. A study of the roles played by these components from 1955 to 1957 has been completed by Kingsland (4). Contemporary studies in New York (1), New Jersey (2), and Canada (6) have consistently pointed to Gibberella zeae as the chief causal agent of stalk rot in these areas. Because of the difference in causal agent, hybrid response to stalk rot in the Northeast is not always correlated with stalk rot response in the corn belt.

Gibberella stalk rot seems to be remarkably subject to climatic variation. Foley (3) and others (5) showed that unbalanced nutrition had a marked influence on stalk rot development. Soil type, drainage, rainfall, maturity, time of first frost, and so forth, have been cited as contributing agents to stalk rot development. Pathologists are now concerned about strain differences among isolates taken from different areas.

The interpretation of stalk rot reaction in hybrid corn is further confused by the methods that various agencies use in accumulating the data. To be truthful, stalk rot has not been strictly defined. The plant pathologist thinks of it in terms of breakdown of fundamental tissues. The agronomist thinks of the disease in terms of stalk lodging, that is, breakage below the ear, as opposed to root lodging. Often there is little distinction made between stalk lodging due to stalk rot and lodging due to corn borer infestation and activity. Evaluation of stalk lodging is not a standardized procedure. Many agronomists count broken stalks; others use a pushing technique to which all standing stalks at harvest time are subjected. A more severe test is one wherein a lusty kick is given the corn plant just above ground level. All these tests are accomplished at the time of harvest, usually 8 to 12 weeks after anthesis. There is little wonder, then, that stalk rot data on corn hybrids in the Northeast provide a controversial issue. On the one hand pathologists are interested in the extent of tissue damage, while on the other

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hand agronomists are interested in what may be spoken of as "standability," that is, the ability of the corn stalk to stand erect, which may be affected for reasons other than poor root support.

In March 1958 the 13th Northeastern Corn Improvement Conference laid plans to investigate stalk rot of corn on a regional basis. Six inbred lines were chosen to represent a series ranging from early to late and susceptible to resistant. Isolates of Gibberella zeae were chosen and sent to C. W. Boothroyd of Cornell, who prepared toothpick culture inoculum. Seed of the inbred lines was distributed to H. L. Everett of Cornell. (Each participant was free to study any number of additional lines he cared to enter.)

For the pathologists, inoculations were to be made at 50 percent silk by puncturing the stalk 6 to 10 inches above ground and inserting a prepared toothpick. Readings were to be taken 6 weeks after inoculation. A uniform method of taking data was suggested. Stalks were to be split at right angles to the protruding toothpick and a visual rating assigned to the extent of internal rot. The rating system was: 0.5, 1.0, 1.5, ... 3.5, and 4.0, where 0.5 represented 12.5 percent or one-eighth of the internodal tissue discolored and 4.0 represented 100.0 percent or complete discoloration of the internodal tissue. In the case of more than one unit of internodal spread, the rating system was to continue 4.5, 5.0, and so forth. Additional methods could be employed at individual discretion and the gathering of supplementary data was encouraged. (The data to be gathered embraced the classical concept of "diseased tissue" rather than the practical concept of "standability.")

Because of unusual weather during 1958, the test conducted at University Park, Pennsylvania was one of the few from which data were taken. The data are presented here because they do represent the local response of the six inbreds during an unusually cool wet season.

Table 1 summarizes the general field data. Six replicates were planted on an eight-row strip in such a way that replicates 1, 2, 3 and 4 were continuous 50-hill plots in rows 1-6, whereas replicate 6 represented four (2 x 25 hill) plots in rows 1-8, and two 50-hill plots in rows 7 and 8. Replicate 5 occupied rows 7 and 8 of the eight-rowed plot. The position of each inbred in each replicate was randomized.

Table 1 also shows the reaction of the various inbreds to emergence from the cold wet soil prevalent throughout the spring.

THE STALK-ROT EXPERIMENT

A New York isolate of Gibberella zeae was compared with one from Pennsylvania. Replicates 1, 3 and 6 were used for the Pennsylvania strain and 2, 4, 5 for the New York strain. In each replicate plants 1, 3, 5, 7, and so forth, were inoculated; plants 2, 4, 6, 8 acted as non-inoculated checks. A few weak atypical plants were discarded along with an occasional outcross revealed by its hybrid vigor.

In taking data, all the replicates of each inbred were considered in the order 1, 2, 3, 4, 6, and 5. Stalks of inoculated and non-inoculated plants were split from ear node to crown and the following data recorded:

1. Stalk rot according to rating system.
2. Actual measurement in mm of extent of stalk rot.
3. Actual measurement in mm of internode length.
4. Actual measurement in mm of dry or non-watersoaked pith.
5. Presence of crown rot and its origin (crown or brace roots).
6. Readings confounded by corn borer infestation and therefore unusable.

Table 2 summarizes the data screened from the total number of plants examined. Corn borer infestations were largely responsible for discarded units[2]. Occasionally poorly chosen inoculation sites led to disqualification of units. These cases were very few.

Non-inoculated plants were uniformly healthy except for the few exhibiting crown rot. In only two cases was crown rot credited to brace root infection; all others originated below the brace root level. In no case did crown rot of inoculated plants exceed that of non-inoculated plants.

Not a single unit examined revealed stalk rot advancing into adjacent non-inoculated internodes.

[2] A unit includes one inoculated plant and its adjacent non-inoculated check.

Table 1. Summary of general field data for six inbred lines of uniform stalk rot trial planted May 18, 1958, University Park, Pennsylvania.

Inbred	Final Stand by Replicate 1	2	3	4	5	6	Seed Planted	Total Stand	Inoculation Date	Readings Taken
Oh 26	12	15	8	15	8	12	300	70	Aug. 7	Sept. 17
Oh 51A	29	29	33	28	37	33	299	189	" "	" 18
Ia Os 420	30	44	36	37	37	36	300	220	" "	" 19
Ind 38-11	43	37	36	39	35	38	300	228	" 25	Oct. 1
Ia L317	26	31	35	27	38	25	300	182	" "	" 2
Oh 07	23	20	23	30	22	22	300	140	" "	" 3

Table 2. Gross summary of data from uniform stalk rot trial.

Inbred Line	Number of Usable Units for Stalk Rot Comparison: Pa. Strain Rep 1	Pa. Strain Rep 3	Pa. Strain Rep 6	Pa. Strain Total	N.Y. Strain Rep 2	N.Y. Strain Rep 4	N.Y. Strain Rep 5	N.Y. Strain Total	Units Discarded No.	Units Discarded %	Crown Rot Percentage of All Plants
Oh 26	3	4	6	13	7	8	4	19	5	13.51	0.0
Oh 51A	12	12	13	37	11	13	17	41	16	17.02	8.9
Ia Os 420	14	18	18	50	22	20	18	60	0	0	4.09
Ind 38-11	19	17	17	53	20	18	11	55	11	9.24	0.0
Ia L317	12	18	13	43	15	14	20	49	2	2.12	0.0
Oh 07	11	12	11	34	11	16	10	37	1	1.38	1.42

TREATMENT OF DATA FROM USABLE UNITS

Three measurements of each inoculated plant were recorded. The rating system is strictly a matter of personal judgment and, because of its implications, the data could reasonably vary among individuals gathering it. One could calculate the personal error factor against the actual measurement of method two ("y").

When the corn stalks were split, there appeared to be a difference among individual plants in the amount of non-water-soaked (dry) pith exposed. Water-soaked pith seemed to be greatest near the nodal plates, perhaps creating the illusion that internodes were shorter than they actually were. This illusion might influence visual rating. It was decided, therefore, to measure the non-water-soaked pith as it appeared upon splitting the stalk. It was finally observed, however, that the amount of watersoaked pith was more characteristic of the inbred lines than of individuals within a line. Ia Os 420 and Oh 07 were characteristically less water-soaked than the other inbreds studied. Both are quite susceptible to stalk rot.

For convenience in further discussion, the data are classified as follows:

(a) x value - rating system 0.5, 1.0, 1.5, and so forth.

(b) y value - actual measurement: 100x total stalk rot/total internode length (Method 2).

(c) z value - actual measurement: 100x total stalk rot/length dry pith.

Correlation values were calculated for xy, xz, yz for each inbred. The results are presented in Table 3.

Table 3. Correlation values between each method of expressing stalk rot data for each inbred line and each fungus strain tested.

Inbred Line	Fungus Strain	Reps Included	Number Observations	Correlation Values rx.y	rx.z	ry.z
Oh 26	Pa.	1.3.6	13	.891	.851	.960
	N.Y.	2.4.5	19	.791	.771	.959
Oh 51A	Pa.	1.3.6	37	.904	.908	.973
	N.Y.	2.4.5	41	.941	.937	.973
Ia Os420	Pa.	1.3.6	50	.877	.895	.950
	N.Y.	2.4.5	60	.956	.926	.996
Ind 38-11	Pa.	1.3.6	53	.983	.982	.988
	N.Y.	2.4.5	55	.937	.915	.976
Ia L317	Pa.	1.3.6	43	.966	.957	.981
	N.Y.	2.4.5	49	.950	.943	.973
Oh 07	Pa.	1.3.6	34	.924	.919	.945
	N.Y.	2.4.5	37	.898	.897	.959

There appear to be two conclusions to be drawn from Table 3. 1) For this observer, the visual rating system is quite satisfactory. A larger number of observations for Oh26 would, of course, be desirable. It would appear that 35 to 50 observations on any inbred line are sufficient. 2) The possibility of being confused by the extent of dry or non-water-soaked pith is not very great[3].

The comparative performance of the inbred lines and the fungus strains used in the experiment must also be examined. The data used in assembling Table 4 were used as a statistical basis. For visual analysis the method of arrangement in Table 4 is as follows:

[3] The determination of differences in pathogenicity among isolates may require closer scrutiny than visual ratings (see Table 4).

Table 4. Summary table of field data.

Inbred Line	Value Used	Fungus Isolate	% of Plants in Classes of Stalk Rot								Group Size	% Below Midpoint	Test of Significance for Isolates
			0.5[a] 0-19[b]	1.0 20-31	1.5 32-44	2.0 45-57	2.5 58-69	3.0 70-81	3.5 82-93	4.0 94-100(+)[c]			
Oh 26	x	Pa.				7.7	7.7	38.4	30.8	15.4	13	7.7	-
		N.Y.				10.5		63.1	26.3		19	10.5	
	y	Pa.				15.4	30.8	53.9			13	15.4	-
		N.Y.				10.5	36.8	31.5	21.0		19	10.5	
	z	Pa.				7.7	15.4	30.8	30.8	15.4	13	7.7	-
		N.Y.					15.8	15.8	52.6	15.8	19	0.0	
Oh 51A	x	Pa.	16.2	5.4	21.6	29.7	10.8	8.1	2.7	5.4	37	72.9	-
		N.Y.	14.4	4.8	7.2	28.8	9.6	26.4	7.2		41	55.2	
	y	Pa.	2.7	24.3	40.5	10.5	13.5	5.4	2.7		37	78.0	0.05
		N.Y.		16.8	24.0	24.0	26,4	7.2			41	64.0	
	z	Pa.		8.1	37.8	18.9	13.5	13.5	2.7	5.4	37	64.8	-
		N.Y.		9.6	12.0	21.6	16.8	26.4	9.6	2.4	41	43.2	
IaOS 420	x	Pa.				2.0	8.0	18.0	28.0	44.0	50	2.0	-
		N.Y.		3.4		3.4	6.8	30.6	25.5	32.3	60	6.8	
	y	Pa.			2.0	4.0	18.0	34.0	42.0		50	6.0	-
		N.Y.		3.4		3.4	13.6	47.6	30.6	3.4	60	6.8	
	z	Pa.				6.0	8.0	24.0	34.0	28.0	50	60.0	-
		N.Y.			3.4	3.4	3.4	28.9	42.5	20.4	60	6.8	
Ind 38-11	x	Pa.	15.2	28.5	9.5	30.4	5.7	7.6	1.9	1.9	53	83.7	<0.05
		N.Y.	25.2	28.8	16.2	18.0	7.2	1.8	1.8		55	88.2	
	y	Pa.		17.1	41.8	30.4	7.6	1.9	1.9		53	89.3	0.05
		N.Y.		39.6	37.8	16.2	3.6	1.8			55	93.6	
	z	Pa.		5.7	30.4	32.3	17.1	11.4	1.9	1.9	53	68.4	0.01
		N.Y.		9.0	50.4	27.0	7.2	3.6	1.8		55	86.4	
Ia L317	x	Pa.	4.7	7.0	11.7	9.3	7.0	23.3	25.6	11.7	43	32.7	-
		N.Y.	4.0	10.0	6.0	18.0	22.0	16.0	20.0	2.0	49	38.0	
	y	Pa.		9.3	16.3	11.7	18.6	32.6	9.3	2.3	43	37.3	-
		N.Y.		6.0	20.0	26.0	22.0	20.0	4.0		49	52.0	
	z	Pa.		4.7	14.0	14.0	7.0	18.7	30.3	11.7	43	32.7	-
		N.Y.		4.0	12.0	22.0	18.0	16.0	18.0	8.0	49	38.0	
Oh 07	x	Pa.				2.9	2.9	14.7	14.7	65.8	34	2.9	-
		N.Y.				2.7		18.9	27.0	51.3	37	2.7	
	y	Pa.				2.9	8.8	29.4	58.8		34	2.9	-
		N.Y.				5.4	5.4	37.8	40.5	10.8	37	5.4	
	z	Pa.				2.9	2.9	17.6	32.3	44.1	34	2.9	-
		N.Y.				2.7	8.1	24.3	29.7	35.1	37	2.7	

a= rating system for "X" values.
b= percentage classes for "Y" and "Z" values.
c= "Z" values sometimes exceed 100%.

1. For each inbred the percent of plants falling in each classification was calculated to the nearest decimal. For the "y" and "z" values, percent classifications were chosen that would reasonably correspond with classes in the rating system.
2. It was assumed that the more virulent isolate of Gibberella zeae would allow the least number of observations below mid-point of the scale. This "least" number became the basis of comparison between inbred lines and between isolates of the pathogen.
3. The actual data were compared statistically using the "t" test for "groups with different numbers of individuals."

A study of Table 4 reveals the following points with respect to data based solely on discoloration and disintegration of the internal fundamental tissue.

1. (a) Oh 26, Oh 07 and Os420 were quite susceptible whereas Ia L317, although classed as susceptible, averaged 38 percent of its population in the resistant end of the scale. The position of Oh 26 is based on too few observations.
   (b) Ind 38-11 and Oh 51A were resistant although Oh 51A contained an average of 36 percent of its population in the susceptible end of the scale.
   (c) For a rapid visual analysis of the data, calculation of the percent of plants below midpoint of the scale is quite accurate.
2. It would appear that any inbred line of the six studied could be improved by inoculation and reselection.
3. The data have not revealed a clear-cut superiority in pathogenicity of the Pennsylvania isolate over the New York isolate.
4. Statistical differences in pathogenicity between the two isolates were indicated only with the resistant inbred lines. The data also indicate that the visual rating system may not be sufficiently accurate to detect differences in pathogenicity.

In a final analysis the "x" values -- and the "y" values also -- for each fungus isolate were averaged for each inbred. By paired comparisons among and between resistant and susceptible inbred lines, the following facts were deduced.

1. The differences between inbreds were significant at either the 0.05 or 0.01 levels for either the averaged "x" or averaged "y" values.
2. The order of the six inbred lines from most resistant to most susceptible was: Ind 38-11, Oh 51A, Ia L317, Oh 26, Ia Os 420 and Oh 07.

The position of Oh 26 might not be tenable if more observations were considered.

DISCUSSION

The three early inbred lines, Oh 51A, Oh 26 and Ia Os 420, are approximately 10 to 12 days ahead of Oh 07, Ind 38-11 and Ia L317. The timeliness of inoculation and of data taking theoretically smoothed out differences due to maturity between the two groups. Under University Park conditions it could be postulated that the early lines underwent the duration between inoculation and data taking at temperatures a few degrees above the corresponding duration for the late lines. Temperature data were not recorded during the experiment. Aside from this postulated difference in temperature we have to assume comparable conditions for all inbreds.

It is emphasized again that the data of this experiment are concerned with discoloration and degeneration of the internal tissue. No attempt was made to relate the influence of tissue rot on stalk strength, that is, "standability." Of the many cooperating groups in the Northeast, only four submitted data and these data reflected the exactly opposite approach to the problem, namely, stalk strength (standability) as determined by the pushing technique, irrespective of the cause of weakening, whether it was rot, injury, or morphological weakness.

A summary of the standability data is as follows:

Station	Order of performance of inbred lines from most resistant to most susceptible					
1	38-11	Oh 26	Oh 51A	Oh 07	L317	Os 420
2	Oh 07	Oh 26	L317	38-11	Oh 51A	Os 420
3	Oh 26	Oh 07	38-11	L317	Oh 51A	Os 420
4	Oh 26	Oh 07	38-11	L317	Oh 51A	Os 420
University Park[a]	38-11	Oh 51A	L317	Oh 26	Os 420	Oh 07

[a]Data on tissue degeneration -- for comparison.

It is quite obvious that there is considerable agreement on the "standability" of the inbreds but that station variability exists; whether this is a true inbred response or a personal variable is a question not likely to be answered. The disagreement of these data with those collected at the Pennsylvania Station is equally obvious. Tissue degeneration and "standability" are not closely correlated. It appears that stalk rot due to Gibberella zeae in the Northeast exhibits a response parallel with that to Diplodia maydis and Gibberella zeae in Missouri (7). In Pennsylvania, Oh 07 has been found quite useful in that it contributes standability and drought tolerance to its hybrids, whereas the use of 38-11 is decreasing because its hybrids do not stand well or tolerate drought. Oh 51A is used because it makes a satisfactory female single cross with WF9. It is not considered to be extremely susceptible to stalk rot. Os 420 is not used at all and L317 is used only in US13. L317 is considered to contribute weak stalks to its hybrids and is generally replaced with C103. Oh 26 is used in Ohio M15 only. Its use is restricted more by its aphid susceptibility than by its stalk reaction.

One of the striking observations made on the six inbred lines used in this study was the range of stalk rot reactions within each line. The range was not so great in the susceptible inbreds -- less so with Ia L317. It would be an interesting study to reselect the best and poorest elements in two inbreds like L317 and 38-11 and study the reaction of crosses made between them. It seems quite unlikely that the variation observed could be the result of technique alone.

Consider the relationship between crown rot and stalk rot. Some pathologists believe all stalk rot is initiated as crown rot (6). The data indicate that the relationship needs further investigation. Other paths of infection such as brace roots or leaf sheaths may play a role.

It would seem a fruitful study to investigate anatomically whether Gibberella zeae has less effect on the outer rind of a line like Oh 07 than on Ind 38-11 or whether the fungus penetrates the rind at all.

If there is as much variation in outer rind structure within inbred lines as there is variation in amount of internal disintegration by Gibberella zeae, then sub-lining each inbred and selecting for "standability" should prove a worth-while procedure. As a matter of fact, "standability" rather than stalk rot resistance seems to be the desirable character to be sought. Whether one could arrive at a practical end point more expediently with or without inoculation is yet to be demonstrated.

In the disease breeding nursery at University Park, where inbreds of all maturities are planted at the same time, it is difficult to be timely with stalk rot inoculations. Inoculations are usually made just after the latest material has been pollinated. Results are usually read at harvest time and interpretations must always be made with maturity in mind. In spite of this seemingly helter-skelter procedure, stalk rot inoculations always seem to be most impressive on S_3, S_4, and S_5 material. Whether this apparent phenomenon is due to the fact that susceptible material in earlier generations was discarded along with plants or ear cultures susceptible to the Helminthosporium leaf blights is not clearly indicated. It is a well-known fact that marked destruction of leaf tissue predisposes the corn plant to stalk rot.

Undoubtedly stalk rot is a complex problem. Is control of stalk rot the aim of the plant pathologist or the plant breeder? Is it in the domain of both disciplines? If so, what is the best procedure?

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PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION, UNIVERSITY PARK

SOME ROOT-ROTTING FUNGI ISOLATED FROM WESTERN GRASSES[1]

Roderick Sprague[2]

During a hurried trip to Phoenix, Arizona and return to Wenatchee, Washington in late March 1959, several root samples of living grasses were obtained from irrigated lawns and gardens at Phoenix and from desert and range areas in northwestern Arizona, central Nevada and eastern Oregon. The plants collected on March 29-31 were packed in their original soil in new cylindrical containers and watered lightly. The excised roots were washed at Wenatchee on April 2 under a fine stream of tap water for 5 hours in perforated Coors crucibles, plated out on non-nutrient agar and finally transferred to 2 percent PDA. The isolates were grown under north light under common storage, non-refrigerated conditions.

Although some of the specimens suffered enroute from root necrosis caused by Pythium spp. most of them arrived in good condition. The desert materials, excluding the damping-off or root necrosis, were comparatively free from parasitic fungi. A number of parasitic and weakly pathogenic fungi were isolated including the following unreported host ranges by States:

CURVULARIA LUNATA on: Eragrostis mexicana, Phoenix, Ariz.; Lolium multiflorum, Phoenix, Ariz.; Tridens pulchellus, west of Bagdad, Ariz.

CURVULARIA GENICULATA on: Hordeum brachyantherum, Haines, Oreg.

FUSARIUM ACUMINATUM on: Bromus rubens, west of Bagdad, Ariz., Mercury, Nev.; Festuca octoflora, 6 miles north of Congress, Ariz.; Hilaria rigida, 6 miles north of Congress, Ariz.; Hordeum brachyantherum, Haines, Oreg.; Lolium multiflorum, Phoenix, Ariz.; Oryzopsis hymenoides, Tonopah, Nev.; Tridens pulchellus, west of Bagdad, Ariz.

F. OXYSPORUM on: Hilaria rigida, 6 miles north of Congress, Ariz.; Oryzopsis hymenoides, Tonopah, Nev.

F. POAE on: Bromus rubens, west of Bagdad, Ariz.

PYTHIUM DEBARYANUM on: Festuca octoflora, 6 miles north of Congress, Ariz.

P. GRAMINICOLA on: Aristida pansa west of Bagdad, Ariz.; Bromus rubens, Mercury, Nev.; Lolium multiflorum, Phoenix, Ariz.

P. ULTIMUM on Eragrostis mexicana, Phoenix, Ariz.

RHIZOCTONIA SOLANI on: Eragrostis mexicana, Phoenix, Ariz.; Lolium multiflorum, Phoenix, Ariz.

Some of the common saprophytes associated with these species were: Alternaria spp., Bispora sp., Brachycladium spiciferum, Fusarium equiseti, a few Mucorales, Phoma herbarum, Pythium vexans, one culture of Rhizoctonia albida and a flat-growing fungus perhaps near Thielaviopsis.

Except for the Pythium spp. and Rhizoctonia solani, none of the secondary rootrots and seedrots mentioned are recognized as being very destructive, when taken individually. Of all the isolations that were made from these collections, only Rhizoctonia solani on Cynodon dactylon from Arizona had been reported from the western area.

WASHINGTON AGRICULTURAL EXPERIMENT STATION, PULLMAN

[1] Information series paper, Washington Agricultural Experiment Stations, Pullman. Project 449.

[2] Plant Pathologist, Tree Fruit Experiment Station, Wenatchee, Washington.

STICKERS FOR FUNGICIDAL SPRAYS IN THE TROPICS[1]

Chas. S. Reddy and R. G. Davide[2]

Summary

A technic was developed for measuring the amount of spray remaining on glass slides after each artificial rain in a series. The technic was the addition of 1 ml of a dilute solution of iodine-stained starch grains to each 400 ml of spray to be tested. Tests were made by spraying glass slides, held at a 45° angle, to the run-off stage, drying, and counting the purple starch grains in the low-power field of the compound microscope. Similar counts were made after each artificial rain. The relation of these counts to the first count showed the proportion of spray remaining after each successive rain. By this method, recommended sticker dosages were found too low to be of much value in the tropics. When tested at efficient dosages, the best stickers were those which contributed the greatest retentiveness to the sprays after at least six artificial rains and which reached efficiency soonest after spray application. The best and cheapest sticker was found to be natural rubber latex, which is easily obtained in the tropics. The synthetic latex, PEPS, was almost as good. The validity of the mechanical technic was confirmed by biological inhibition tests, and glass slides were found to be as reliable as leaf tissue.

Foliage diseases are especially severe in the Philippines, because of high humidity and frequent rains throughout much of the year. At the turn of the century, coffee rust (*Hemileia vastatrix* Berk. & Br.) stopped the growing of a thriving valuable crop, not only in the Philippines but also in Indonesia, Ceylon, and India. Coffee production (arabica type) is again being increased. Its future depends on economical disease control. Better spray stickers are badly needed.

Spraying to control foliage diseases in the tropics is often unprofitable because frequent rains wash away the fungicide and make necessary many applications at short intervals. It was hoped that stickers could be added to the sprays to make them stay on the foliage longer and remain effective longer. When spray materials are evaluated under conditions of frequent rains, the character of adhesiveness is often more important than the chemical itself. The new spray materials are less phytotoxic than Bordeaux mixture, and, when applied often, are more effective. However, Bordeaux mixture is almost invariably the choice because it sticks to the foliage better. The objective, therefore, is to find a sticker that will make possible in the tropics the economic use of sprays, such as carbamates, phthalimides, and quinones, that have been found superior in regions of lower rainfall where adhesiveness is not so important.

MATERIALS AND METHODS

The first step toward the solution of the sticker problem was to develop a quick laboratory technic to determine the percentage loss of spray in each successive artificial rain. Weighing with the analytical balance was impractical because weight losses were very small in relation to uncontrolled humidity. Use of an indicator seemed more feasible, so a small quantity of India ink was added to 400 cc of each experimental spray. It was thought that sprayed glass microscope slides could be viewed with the low power of the compound microscope and the insoluble carbon particles counted, but the extreme range in particle size of India ink made this impossible. However, the method proved successful when iodine-stained starch grains were

[1] Journal Paper No. J 3422 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 858.

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substituted for India ink. The dilute starch solution was so adjusted that, when 1 cc was added to 400 cc of spray and when slides, held at a 45° angle, were sprayed to the drip point, the low-power field of the compound microscope showed 40 to 48 starch grains. Counts of starch grains before and after rain indicated the percentage of spray remaining on the slide after each rain. Three fields were counted on each slide, and duplicate experiments were averaged for each figure shown in the data. Only two promising spray materials were used, namely zineb (Parzate or Dithane Z-78; zinc ethylene bis dithiocarbamate) and captan (Orthocide 40W; N-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide), each at the recommended rate of 2 pounds per 100 gallons. Because of its inherent sticking characteristic, Bordeaux mixture was also evaluated. To compare a number of stickers at a single rate, duplicate experiments were performed in which each sticker was added at the proper rate to 400-ml portions of spray containing the starch indicator. One portion, to which no sticker was added, served as a control. Seven slides placed on a rack permanently slanted to 45° were sprayed from one beaker by an electric sprayer to the point of runoff. Sets of 7 slides representing each sticker or dosage were sprayed or washed in series so as to economize on drying time. After drying, 1 of the 7 slides was used to determine the number of starch grains per low-power microscopic field. After drying for at least 3 hours the remaining 6 slides were washed with 20 ml of water from the electric sprayer. The starch grains on a second slide were then counted. This procedure was repeated with the remaining 5 slides to obtain data for 6 washings (artificial rains). The same procedure was used for the control and for each of the stickers. Repeating the experiment with successively higher sticker rates showed the relative value of the stickers at different rates and the optimum practical dosage at which each sticker should be used. This technic can also be used with the optimum sticker dosage to show the drying time required for each sticker to become fully efficient.

The following stickers were used:

PEPS--Polyethylene polysulfide, 56 percent. Spencer Chemical Co., Dwight Bldg., Kansas City, Missouri.

Tenac--Shell Chemical Corporation, 460 Park Ave., New York 22, New York.

Shellestol--Shell Chemical Corporation.

Ortho Spreader Sticker--California Spray Chemical Company, Richmond, California.

Macondray Spreader Sticker 78--Macondray Company, Manila, P. I.

Goodrite X75--Goodrich Chemical Co., 324 Rose Bldg., Cleveland 15, Ohio.

Goodrite SDD--Sodium dimethyldithiocarbamate, 40 percent. Goodrich Chemical Co., 324 Rose Bldg., Cleveland 15, Ohio.

Triton B-1956--Modified phthalic glycerol alkyd resin. Rohm and Haas, Washington Square, Philadelphia 5, Pennsylvania.

Du Pont Spreader Sticker -- Sodium sulfates mixed long chain alcohol fatty acids and esters, 88 percent. E. I. du Pont de Nemours & Company, Wilmington, Del.

Many biological experiments could be conducted to help interpret data obtained by this technic. Inhibition of the fungus Curvularia inaequalis (Shear) Boed. on potato-dextrose agar in Petri dishes was used to confirm the reliability of the indicator count as a measure of the amount of spray remaining after each washing.

In these tests cover slips of 18 mm diameter were used instead of slides, but twice as many, so that one slide could be used for the mechanical test and one for the inhibition test at each step. Spores were suspended in agar, plates poured, and a cover slip placed with the spray side down in the middle of each plate. At 16 hours the inhibition zone was determined by three diameter readings of the circular area in which spore germination was inhibited. The duplicate cover slip was used for the starch-iodine mechanical test to determine the percentage of spray remaining after any particular washing. The counterpart for each of these percentages was the relation of the inhibition area (cm^2) to that of the control (no washing).

EXPERIMENTAL RESULTS

Dosage data on the efficiency of spray stickers were determined by the starch-iodine method. The results of an experiment in which three stickers were used in zineb spray are presented in Table 1. PEPS and Tenac are commercial stickers. The natural rubber latex was diluted to 14 percent latex, and contained ammonia to prevent coagulation.

The data in Table 1 show that the low dosage of sticker (1/2 pint per 100 gallons) is of

Table 1. Percentages of spray material remaining after each of six washings when latex (14 percent), PEPS, and Tenac stickers were used at the indicated rates per 100 gallons Parzate spray[a].

Treatment	Percentage of spray retained after washing no. 1	2	3	4	5	6
	1/2 pint sticker per 100 gallons					
Parzate + latex	77	57	41	32	25	19
Parzate + PEPS	69	53	37	26	21	15
Parzate + Tenac	68	47	31	23	18	13
Parzate alone	63	43	30	22	16	12
	1 pint sticker per 100 gallons					
Parzate + latex	83	61	51	36	24	17
Parzate + PEPS	73	58	45	33	24	15
Parzate + Tenac	70	57	45	30	19	13
Parzate alone	58	39	27	19	13	7
	2 pints sticker per 100 gallons					
Parzate + latex	86	78	67	58	45	38
Parzate + PEPS	86	74	63	57	45	35
Parzate + Tenac	84	72	58	43	33	27
Parzate alone	63	51	41	28	20	13
	3 pints sticker per 100 gallons					
Parzate + latex	85	77	63	54	47	39
Parzate + PEPS	83	72	63	55	40	38
Parzate + Tenac	74	60	54	46	35	31
Parzate alone	63	42	30	23	15	10

[a]Data are averages of two experiments.

little value in retaining spray material through successive rains. The highest dosage (3 pints) was the most effective. These data suggested testing higher dosages, so an experiment was conducted with dosages of 1/2 to 4 pints per 100 gallons. The data are presented in Figure 1.

Figure 1 again shows that the usually recommended small dosages of sticker were of little value, and that the 3-pint dosage was the most efficient. The 4-pint dosage was not very different from the 3-pint dosage, and in this case was less effective. Table 1 and Figure 1 agree in showing that, after 6 artificial rains, the 1/2-pint dosage of latex sticker resulted in retention 1 2/3 times that of the check; 1 pint in 2 1/2 times, 2 pints in 3 times, and 3 pints in 4 times, as much retention as the check.

Figure 2 shows that benefits were improved when any one of eight different stickers was used at higher rates than usually recommended. Latex was the best, while Shellestol was shown to be a spreader, not a sticker. Data are from Table 1 and Figure 1, and from two additional experiments.

Figure 3 shows that (nearly) half the spray was lost in the first washing when stickers were used at low dosage (1/4 pt./100 gal.), and less than one-sixth was lost when dosage was 3 pints per 100 gallons. After 6 washes, there was little difference in percentage of spray remaining, whether stickers at low dosage or no stickers were used (14:12). At high dosage, the relation was 34:11 between stickers and no stickers.

Figure 4 shows that, when the starch-iodine technic was used, the data on spray retention are nearly alike in two experiments. The data also show that du Pont Spreader Sticker produced spray retention of only 17 percent when 4 or 8 ounces per 100 gallons were used, but that retention efficiency was increased to 40 percent when the dosage was 3 pints per 100 gallons.

Sticker efficiency data were obtained from experiments using a single dosage (3 pt./100 gal.). Figure 5 shows the percentages of spray retained by different stickers after each of 6 washes. Latex was the best sticker and Shellestol the poorest, but the synthetic latex sticker, PEPS, was almost as good as latex. The data also indicate that, without sticker, more captan than zineb spray was retained. After 6 washes only 10.7 percent of zineb spray remained,

compared with 22.2 percent of the captan spray. Figure 5 also shows that the principal difference in the value of these stickers is their response to the first artificial rain. There is much less difference in response to succeeding rains.

Figure 6 shows that, with either latex or PEPS, 80 percent or more of the spray is retained when rain occurs as early as 1 1/2 hours after application. When Triton B-1956 was used, 4 hours were required to reach this efficiency. Tenac required 10 hours. With no sticker, only 63 percent of the spray was retained when rain was delayed 24 hours. In the tropics, reaching maximum efficiency in a short time may often prevent the loss of a spray.

Parallel experiments were conducted to compare results obtained from the starch-iodine method with those from the inhibition method. The inhibition technic used suspensions of Curvularia inaequalis spores in potato-dextrose agar poured plates. Instead of microscope slides, 18-mm round cover slips were used.

The results of experiments to measure the retention of spray after each of several washings (artificial rains) by two methods are presented in Figure 7. The objective was to compare the reliability of a simple mechanical test (starch-iodine technic) with that of the usual biological inhibition technic, which is more cumbersome.

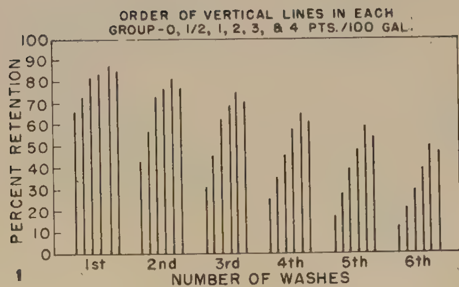
Figure 7 shows that the inhibition test confirmed the validity of the starch-iodine technic for determining the relative retention ability of three spray stickers and a check. Figure 7 shows more consistent curves for the starch-iodine technic than for the biological technic, and the former is probably a more accurate measure of the amount of spray remaining. This could be expected because biological tests usually have a greater number of uncontrolled factors than do mechanical tests. In one of the two experiments graphed in Figure 7, the inhibition method seems to exaggerate the amount of spray residue held by the stickers, especially where the starch-iodine technic shows about 30 percent or more of the original spray remaining.

Glass cover slips and leaf disks of the same size were compared for spray retention. The disks were taken from chlorophyll-deficient areas of a variegated plant (arrowroot) so that the stained starch grains would be easily visible for counting with the microscope. Figure 8 shows that the cover slip data are almost identical with the leaf disk results. In this experiment comparison between the starch-iodine and the inhibition methods indicated that percentages of spray retention from the inhibition method remained larger than percentages from the mechanical method. Fungicidal efficiency did not decrease so rapidly as the loss of spray material. Fungicidal efficiency rating usually decreases rapidly only after less than 30 percent of the original spray remains.

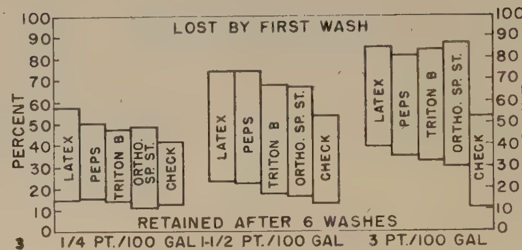
DISCUSSION

Data needed to predict the lowest amount of spray that is efficient in disease protection, are still being recorded from biological experiments in progress. Coffee plants not sprayed and sprayed, with and without latex sticker, have been inoculated with Cercospora coffeicola after successive rains to determine how much longer the sticker makes the spray efficient. Also, the value of a good spray sticker, natural rubber latex, added to Bordeaux and zineb is being determined in a 4-year-old commercial planting of arabica coffee at Matutum in Mindanao. The inhibition technic showed that decreases in inhibition zone area are small as the percentage of original spray decreases until about 25 to 30 percent remains, after which the inhibition zone usually decreases rapidly. This result may be interpreted to mean that efficient protection persists until less than 25 to 30 percent of the original spray remains.

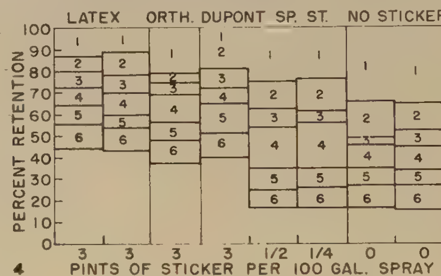
The starch-iodine technic helped to find and evaluate a new spray sticker that appears promising for the tropics. The new sticker is natural rubber latex, which not only is the best sticker but also is common in the tropics around the world. Probably improvements can be made upon the material used in these experiments. The sample that was used had been diluted to 14 percent latex and strained so that bark and other debris had been removed. A little more ammonia was added to the sample to prevent any possible coagulation. The preparation had the appearance and consistency of milk. This dilute form was compared, pint for pint, with other stickers, and proved as good as the best, or better. Latex direct from the rubber tree is double the concentration used in these experiments. Therefore it is reasonable to assume that 1 1/2 pints of this more concentrated natural latex would be equal to 3 pints of PEPS in effectiveness. In the Philippines the cost of the natural rubber latex is much less than that of commercial stickers, and large plantations that would use a sticker in quantity could grow a few rubber trees, which would be the ideal place to preserve sticker until use. Latex taken directly from the tree in a little ammonia water could be used at 1/4 ounce per gallon of spray;


ORDER OF VERTICAL LINES IN EACH GROUP-0, 1/2, 1, 2, 3, & 4 PTS./100 GAL.
PERCENT RETENTION
1st
2nd
3rd
4th
5th
6th
NUMBER OF WASHES

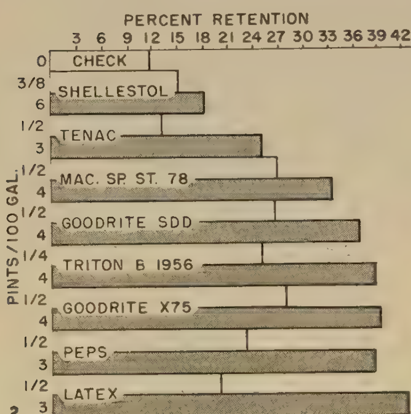
1


LOST BY FIRST WASH
PERCENT
LATEX
PEPS
TRITON B
ORTHO. SP. ST.
CHECK
RETAINED AFTER 6 WASHES
1/4 PT./100 GAL
1-1/2 PT./100 GAL
3 PT./100 GAL

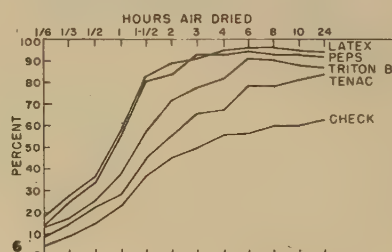
3


LATEX
ORTH. DUPONT SP. ST.
NO STICKER
PERCENT RETENTION
PINTS OF STICKER PER 100 GAL. SPRAY

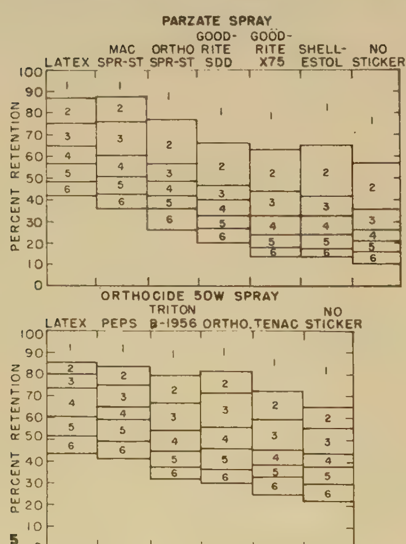
4


PERCENT RETENTION
PINTS/100 GAL.
CHECK
SHELLESTOL
TENAC
MAC. SP. ST. 78
GOODRITE SDD
TRITON B 1956
GOODRITE X75
PEPS
LATEX

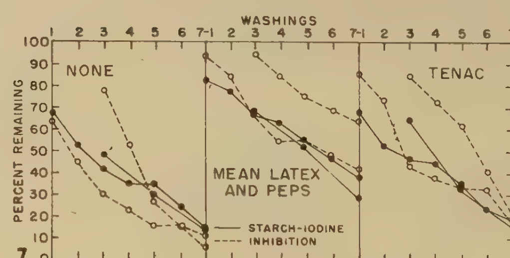
2


HOURS AIR DRIED
PERCENT
LATEX
PEPS
TRITON B
TENAC
CHECK

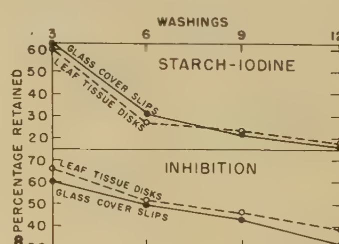
6


PARZATE SPRAY
LATEX
MAC SPR-ST
ORTHO SPR-ST
GOOD-RITE SDD
GOOD-RITE X75
SHELL-ESTOL
NO STICKER
PERCENT RETENTION
ORTHOCIDE 50W SPRAY
LATEX
PEPS
TRITON B-1956
ORTHO.
TENAC
NO STICKER
PERCENT RETENTION

5


WASHINGS
PERCENT REMAINING
NONE
MEAN LATEX AND PEPS
TENAC
STARCH-IODINE
INHIBITION

7


WASHINGS
PERCENTAGE RETAINED
STARCH-IODINE
GLASS COVER SLIPS
LEAF TISSUE DISKS
INHIBITION
LEAF TISSUE DISKS
GLASS COVER SLIPS

8

but it should be diluted in part of the spray water for straining into the spray can or tank to free it from debris that would clog the spray nozzles. If this cheap sticker can double the time that a spray can remain efficient and also shorten the period after application of extreme vulnerability of sprays to washing off by rain, the use of sprays for disease control in the tropics will greatly increase.

PLANT PATHOLOGY, IOWA STATE COLLEGE, AMES, IOWA, AND U. P. COLLEGE OF AGRICULTURE, LOS BANOS, LAGUNA, P. I.

Legends for Figures 1-8

FIGURE 1. Percentage of zineb spray that remained after each wash (artificial rain) when latex sticker was used at different rates. Order of vertical lines in each group 0, 1/2, 1, 2, 3, and 4 pints/100 gallons.

FIGURE 2. Effect of low and high dosages of several stickers on percentage zineb spray retention after six washes.

FIGURE 3. Effect of 1/4, 1 1/2, and 3 pints sticker per 100 gallons on zineb spray retained during six washes. Top of each block shows the percentage of spray remaining after the first wash. Bottom of each block shows percentage spray retained after six washes.

FIGURE 4. The percentage of Orthocide 50W spray remaining after each of six washes in two experiments on latex, Ortho Spreader Sticker, and Du Pont Spreader Sticker, using the starch-iodine method for determining retention.

FIGURE 5. Effect of different spray stickers on spray retention during six washes (stickers used at 3 pints/100 gallons.)

FIGURE 6. Comparison of time-requirements of four stickers to become effective (high percentage retention) after spray application. (Each slide electric-spray washed with 10 cc water.)

FIGURE 7. Effect of stickers on spray retention as measured by two methods: 1) starch-iodine, solid line; 2) growth inhibition, broken line.

FIGURE 8. Mean percentage spray retention on glass cover slips and leaf tissue disks measured by two methods.

POLYBUTENES -- A PROMISING CONTROL FOR POWDERY MILDEW

R. W. Fisher[1]

Abstract

Five percent emulsions of polybutenes were applied to powdery mildew-susceptible cucumber plants in a greenhouse. The sprays provided excellent protection from infection for at least a month. The polybutenes also showed promise in eradicating established infections on roses and cucumbers.

During the course of tests using polybutenes to control the the two-spotted spider mite, Tetranychus telarius (L.), on greenhouse crops[2], it was noted that established infections of powdery mildew, Sphaerotheca pannosa (Wallr.) Lév., on the leaves of roses were removed and that no new infections developed for several weeks after spraying. This suggested that polybutenes of proper viscosity might prevent and/or eradicate powdery mildews on other plants as well.

MATERIALS AND METHODS

Tests were conducted at the Ontario Horticultural Experiment Station, Vineland Station, Ontario, in a greenhouse which contained cucumbers being used in the selection of powdery mildew-resistant strains. The pathogen, Erysiphe cichoracearum, had been intentionally introduced into the house and leaves of susceptible varieties were soon heavily infected.

In the first test, designed to assess the value of the polybutene in preventing infection, 5 percent emulsions of Indopol L-10 and Indopol H-50[3] were each applied before infection to groups of 6 plants of a susceptible variety, all parts of the plants being thoroughly covered. Adjacent unsprayed plants were left as a control.

In the second test, to assess the value of the polybutenes against established infections, only one side of individual plants was sprayed with polybutenes L-100, H-35, H-100, H-300, and H-1500[3] as 5 percent emulsions. The leaves before treatment were completely covered with mycelium. All emulsions were prepared as 30 percent stocks with Atlox G-3300[4] emulsifier, using a high speed electric drill fitted with a stirring rod. These stock emulsions were then diluted as required.

RESULTS

After 18 days, the leaves on the plants sprayed with L-10 showed only a few infections 1 to 5 mm in diameter, widely spaced on the leaves. Even after a month the colonies had spread very little (Fig. 1). Polybutene H-50 prevented infection completely for over 3 weeks and gave good protection for over a month. Leaves and stems on untreated control plants, meanwhile, became completely covered with mycelium (Fig. 2).

In the second test, control of the powdery mildew on the sprayed halves of plants was sharply defined even after 3 weeks of a constant bombardment by spores from the unsprayed halves and from adjacent unsprayed plants. The polybutenes matted the mycelium down so that the leaves no longer appeared white but regained their normal green colour, with chlorotic areas at the sites of infection.

In both tests all sprayed plants continued to grow and produce flowers and fruit with no obvious retardation or phytotoxicity.

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[1] Entomologist, Entomology Laboratory, Research Branch, Vineland Station, Ontario, Canada.

[2] R. W. Fisher, J. Econ. Entomol. (in press).

[3] Indopol Polybutenes, Products of the Amoco Chemical Corp., 910 South Michigan Ave., Chicago, Illinois, distributed in Canada by the R. J. Brown Co., 150 Bronoco Ave., Toronto, Ontario. Viscosities SSU at 210°F: L-10, 40.6; L-100, 93.8; H-35, 375; H-50, 540; H-100, 1070; H-300, 3000; H-1500, 15000.

[4] Product of Atlas Powder Co., Brantford, Ontario, Canada.

FIGURE 1. Prevention of infection by powdery mildew on cucumbers sprayed with L-10 polybutene. Note the heavy mass of mycelium on the unsprayed control on the left and the fewer and smaller colonies on the treated leaves to the right.

FIGURE 2. Prevention of infection by powdery mildew on cucumbers sprayed with H-50 polybutene. Note the virtual absence of mycelium on the treated leaves to the left and the heavy mat of mycelium on the adjacent and untreated plants to the right.

DISCUSSION

When the powdery mildew finally attacked the treated leaves, the colonies were small and widely scattered and appeared to be restricted to areas of the leaf between the spray droplets, or to new surface produced by the growth of the leaf and the breaking up of the polybutene film. The polybutene most likely traps spores settling on it and prevents their successful germination. With established infections, microscopic examination showed that the polybutenes wet the mycelium and flattened it down upon the leaf surface, thus making the mycelium translucent and permitting the green of the leaf to be visible again. Whether the action of the polybutenes is purely mechancial or in part chemical remains to be investigated. Further investigation is needed also to establish which are the most suitable polybutenes, the best formulations, and the most satisfactory timing of applications. However, at this time it appears that the polybutenes show promise in the prevention and/or eradication of powdery mildew on cucumbers grown in the greenhouse.

CANADA DEPARTMENT OF AGRICULTURE, RESEARCH BRANCH

THE EFFICACY AND LIMITATIONS OF HEXACHLOROBENZENE FOR THE CONTROL OF ONION SMUT[1]

Ruben Duran and George W. Fischer[2]

Abstract

The results of 2 years of fungicide trials in the field and in the greenhouse in eastern Washington for control of onion smut, Urocystis colchici (Schlecht.) Rabenh. (U. cepulae Frost), indicate that this disease can be controlled with hexachlorobenzene (HCB), a fungicide heretofore considered highly specific for certain wheat smuts. In soil naturally infested with the onion smut fungus, untreated onion seed resulted in as much as 92 percent smutted seedlings, whereas onion seed pelletized with 80 percent HCB produced as little as 0.41 percent smutted seedlings. It was found that proprietary formulations of HCB vary greatly in efficacy of smut control, and some are markedly phytotoxic. The original HCB proprietary formulation, a product known as "Anticarie," has been consistently excellent in the control of onion smut and consistently free of phytotoxic effects upon the seedlings. The comparative efficacy of other fungicides for onion smut control is discussed.

INTRODUCTION

Onion growers near Walla Walla, Washington are beset with various onion diseases, but in recent years smut, Urocystis colchici (Schlecht.) Rabenh. (U. cepulae Frost), has been most destructive. For one reason or another, the growers have rejected previously-established means for the control of onion smut. Although formaldehyde, used either as a soil treatment prior to seeding or by the drip method simultaneous with seeding, has long been known to control onion smut the growers object to its nuisance aspect. Thiram, widely and successfully used elsewhere, has not proven satisfactory in the Walla Walla area. Therefore, it was decided to seek other fungicides that would be effective, safe, practical, and easy to use for the control of onion smut.

Thaxter (4) made the important discovery that onion plants are attacked by the smut fungus only while very small, and that onion seedlings grown in smut-free soil would remain healthy even though they were later transplanted into soil badly infested with smut. One means of controlling onion smut, therefore, is to germinate the seed in smut-free seed beds and later transplant the seedlings into the field. Even if the field soil is heavily infested with the smut fungus, the transplants remain healthy. In some areas, however, growers regard transplanting as tedious, laborious, and costly. As early as 1900, Sirrine and Stewart related that whenever this method of control was suggested to onion growers, the latter immediately objected that, "it is not practical; it is too much labor to transplant onions!" (3).

Nevertheless, transplanting is almost universally practiced by the onion growers in the Walla Walla area. For a long time these growers kept smut under control by this method, but gradually all their soil became infested and they were forced to grow their seedlings in smut-infested soil. As a result, many growers have been plagued with as much as 50 to 75 percent smut in seedlings. This represents a loss in several ways: First, at transplanting time smutty seedlings must be detected and removed to avoid transplanting them to the field since they would not make salable onions even if they survived. Secondly, growers end up with an insufficient supply of seedlings to meet their needs. Thirdly, heavy seeding to offset seedling losses is an additional cost.

Herein are given the results of fungicide tests conducted during the past 2 years under field and greenhouse conditions, in the search for a satisfactory means of onion smut control.

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MATERIALS AND METHODS

Preliminary experiments were conducted in June 1957. Purdy (2) had just discovered that hexachlorobenzene (HCB), a fungicide highly effective and specific for a few wheat smuts, also controls flag smut of wheat, Urocystis agropyri (Preuss) Schroet. He suggested to us personally that this fungicide might also control onion smut since it, too, is caused by a species of Urocystis. In the June 1957 experiments, several popular fungicides were tested, but the most extensive tests were with a proprietary compound containing 40 percent HCB. The fungicides were applied by pelletizing the seed with equal parts by weight of fungicide, and a 4 percent methyl cellulose sticker, after the method of Linn and Newhall (1).

Twenty fungicides, listed below, were tested in this series of experiments:

Anticarie -- 80 percent hexachlorobenzene. H. P. Rossiger Co., Inc.
Anticarie -- 40 percent hexachlorobenzene. H. P. Rossiger Co., Inc.
Bayer 22555 -- 50 percent wettable powder. P-dimethylaminobenzenediazo sodium sulfinate. Chemagro Corp.
BSM11 -- Phenylmercuric acetate 10 percent, potassium 2,4,6-trichlorophenate 50 percent. Buckman Laboratories, Inc.
Captan -- 50 percent dispersible powder. N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide. Standard Oil Development Co.
Chemagro C-272 -- 20 percent wettable powder, composition unknown. Chemagro Corp.
Chemagro D 113 -- 20 percent wettable powder, composition unknown. Chemagro Corp.
Chipman -- 80 percent hexachlorobenzene. Chipman Chemical Co.
Chloropicrin -- trichloronitromethane.
Dyrene -- 50 percent wettable powder. 2,4-dichloro-6-(o-chloroanilino)-s-triazine. Chemagro Corp.
Mylone -- 85 percent wettable powder. 3,5-dimethyltetrahydro-1, 3, 5, 2H-thiadiazine-2-thione. Union Carbide and Carbon Corp.
No Bunt -- 40 percent hexachlorobenzene. Chipman Chemical Co.
No Bunt -- 80 percent hexachlorobenzene. Chipman Chemical Co.
RE 4334 -- experimental fungicide, composition unknown. California Spray-Chemical Co.
Sanocide -- 40 percent hexachlorobenzene. California Spray-Chemical Corp.
Smut Go -- 40 percent hexachlorobenzene. Miller Products Co.
Stauffer -- 40 percent hexachlorobenzene. Stauffer Chemical Co.
TCNA -- 20 percent tetrachloronitroanisole. Columbia Southern Chemical Co.
Terraclor -- 75 percent pentachloronitrobenzene. Mathieson Chemical Corp.
Thiram -- 50 percent wettable powder. Tetramethylthiuramdisulfide. E. I. du Pont de Nemours & Co.

All subsequent field trials for onion smut control were conducted in late summer. Since late August is the normal planting time for onions in Walla Walla it seemed likely that the results of fungicide tests would be most reliable if they were obtained under the same conditions which prevail during the commercial onion-growing season. Also, the onion maggot caused such extensive damage in the preliminary, June 1957, field trials that thereafter all fungicide-treated seed was also treated with various insecticides for onion maggot control. Besides the pelletizing treatment, some of the fungicides were applied in the furrow at the time of seeding; soil fumigants were applied to the soil in advance of seeding as prescribed by the makers of the products.

Replicate plantings were made in all cases, and were randomized in 6- or 7-foot rows, using aliquot samples of seed. The insecticides were dusted in the furrow after the fungicide-treated seed was sown. All of the field experiments were conducted in naturally infested soil.

Data were taken on the entire plots approximately 2 months after seeding. The seedlings were dug carefully and the roots washed with tap water. They were then placed between moist paper towels or in paper bags and refrigerated to avoid desiccation until smut counts could be made.

A determination of the relative efficacy of the treatments in the field was based on the percentage increase or decrease in amount of smut as compared with the untreated checks. For example, if the fungicide treatment included endrin for maggot control, then the check also was treated with endrin. If a particular treatment did not include endrin it was compared with a "non-endrin check."

The dark smut sori (sometimes internal) were detected in lightly infected seedlings by

Table 1. Effect of fungicide and fungicide-insecticide combinations on onion smut control, seedling stands, and vigor, as reflected in average weight of healthy seedlings. Walla Walla, Washington, 1957[a].

Treatment per 3 grams seed replicate	Stand: Number of seedlings	Percent smut	Size: Average weight per healthy seedling (grams)
Check + 375 mg 50% endrin	1,328	37.5	1.0
Check	969	36.4	1.1
3 g 50% captan (P)[b] + 375 mg 50% endrin	1,802	21.7	1.1
*3 g 50% captan (P)	930	11.1	1.0
3 g 40% HCB (P) + 375 mg 50% endrin	1,132	5.8	0.8
3 g 40% HCB (P)	1,342	1.7	1.0
3 g 40% HCB (F) + 375 mg 50% endrin	1,333	5.2	1.0
3 g 40% HCB (P) + 750 mg 50% heptachlor (P) + 375 mg 50% endrin	970	7.6	0.9
3 g 40% HCB (P) + 750 mg 50% heptachlor (P)	1,182	5.7	0.9
3 g 40% HCB (P) + 3 g 50% captan (P) + 375 mg 50% endrin	1,679	12.9	0.8
3 g 40% HCB (P) + 3 g 50% captan (P)	1,631	13.5	1.0
*3 g 40% HCB (P) + 3.6 g 25% Diazinon	971	.41	1.1
*3 g 40% HCB (P) + 6 g 50% DDT	888	1.2	1.1
*3 g 40% HCB (P) + 1.8 g 50% dieldrin	854	2.5	1.0
3 g 50% thiram (P) + 375 mg 50% endrin	1,401	29.6	1.0
3 g 50% thiram (P)	1,548	26.0	1.1
RE 4334 solution sprayed in furrow + 375 mg 50% endrin	1,084	.09	0.7
RE 4334 solution sprayed in furrow	504	1.1	0.3
8 g 50% PCNB (F) + 375 mg 50% endrin	366	2.1	0.8
*8 g 50% PCNB (F)	378	1.5	0.6
3 g 50% PCNB (P) + 375 mg 50% endrin	1,438	.97	0.8
*3 g 20% PCNB (P)	562	1.7	0.6
3 g 20% TCNA (S) + 375 mg 50% endrin	1,416	.91	0.7
*3 g 20% TCNA (S)	533	1.4	0.6
2 oz./bu. 20% Chemagro D113 (S) + 375 mg 50% endrin	1,451	35.0	0.8
*2 oz./bu. 20% Chemagro D113 (S)	933	12.7	1.0
12 g 5% Chemagro C272 (S) + 375 mg 50% endrin	306	.65	0.8
*12 g 5% Chemagro C272 (S)	304	.98	1.0
Chloropicrin (SF) 3 cc/sq.ft. + 375 mg 50% endrin	1,174	10.5	1.4
Mylone (SF) 1 lb./100 sq. ft.	935	32.0	1.2

[a]Based on two checks: endrin or non-endrin. Average of three replications of three 6-foot rows each, except * = average of two replications.
[b](P) = pelletized seed; (F) = furrow application; (S) = slurry application; (SF) = soil fumigation.

Table 2. Effect of fungicide and fungicide-insecticide combinations on onion smut control, seedling stands and vigor, as reflected in average weight. Walla Walla, Washington, 1958.[a]

Treatment per 3 grams seed replicate	Stand: Number of seedlings	Percent smut	Size: Average weight per healthy seedling (grams)
Check	578	92.0	.32
3 g 40% HCB (P)[b] + 1.6 g 25% Diazinon	0	0	--
3 g 40% HCB (P) + 3 g 50% DDT	0	0	--
3 g 40% HCB (P) + 3/4 g 25% endrin	1	100	.20
3 g 40% HCB (F) + 3/4 g 25% endrin	301	50.5	.49
3 g 40% HCB (P) + 2.7 g 15% Guthion	3	0	.50
*1.5 g 40% HCB (P) + 1.6 g 25% Diazinon	24	58.3	.24
*1.5 g 40% HCB (P) + 3 g 50% DDT	9	55.5	.33
1.5 g 40% HCB (P) + 3/4 g 25% endrin	43	60.4	.36
1.5 g 40% HCB (F) + 3/4 g 25% endrin	592	54.0	.47
1.5 g 40% HCB (P) + 2.7 g 15% Guthion	34	47.0	.31
*3/4 g 40% HCB (P) + 1.6 g 25% Diazinon	138	34.7	.32
*3/4 g 40% HCB (P) + 3 g 50% DDT	133	39.0	.38
3/4 g 40% HCB (P) + 3/4 g 25% endrin	152	60.5	.40
3/4 g 40% HCB (F) + 3/4 g 25% endrin	627	64.7	.48
3/4 g 40% HCB (P) + 2.7 g 15% Guthion	229	62.0	.34
3 g 40% HCB (F)	262	60.6	.52
3 g 40% HCB (P)	1	0	.70
1.5 g 40% HCB (P)	26	57.6	.17
3/4 g 40% HCB (P)	155	65.8	.32
*3 g 50% thiram (P) + 1.6 g 25% Diazinon	523	64.8	.50
*3 g 50% thiram (P) + 3 g 50% DDT	572	40.7	.54
3 g 50% thiram (P) + 3/4 g 25% endrin	734	79.0	.40
3 g 50% thiram (P) + 2.7 g 15% Guthion	866	63.9	.50
3 g 50% thiram (P)	708	80.6	.41
*3 g 50% Dyrene (F) + 1.6 g Diazinon	527	58.6	.47
*3 g 50% Dyrene (F) + 3 g 50% DDT	598	44.9	.54
3 g 50% Dyrene (P)	789	85.6	.36
5.09 g 10% Dyrene (F) + 3/4 g 25% endrin	1,096	68.5	.51
5.09 g 10% Dyrene (F) + 2.7 g 15% Guthion	956	63.4	.47
*3% sol. BSM 11 (S) + 1.6 g 25% Diazinon	5	40.0	.50
*3% sol. BSM 11 (S) + 3 g 50% DDT	17	47.0	.35
3% sol. BSM 11 (S) + 3/4 g 25% endrin	41	51.2	.34
3% sol. BSM 11 (S) + 2.7 g 15% Guthion	22	68.1	.14
3% sol. BSM 11 (S)	34	64.7	.31
*3 g 20% TCNA (P) + 1.6 g 25% Diazinon	137	18.9	.39
*3 g 20% TCNA (P) + 3 g 50% DDT	272	3.6	.36
3/4 g 20% TCNA (P) + 3/4 g 25% endrin	497	27.1	.45
1.5 g 20% TCNA (P) + 2.7 g 15% Guthion	740	21.0	.33
3 g 20% TCNA (P)	353	18.4	.61
*1.6 g 25% Diazinon	362	58.2	.47
3 g 50% DDT	519	58.9	.41
3/4 g 25% endrin	776	84.1	.38
2.7 g 15% Guthion	721	82.8	.27
5 mg 50% Bayer 22555	944	78.6	.49

[a]Average of three replications of two 9-foot rows each, except * = average of two replications.
[b](P) = pelletized seed; (F) = furrow application; (S) = slurry application.

holding them against a background of transmitted light. Seedlings were scored as smutty regardless of number or size of sori.

The greenhouse tests were made in much the same way as the tests in the field except that the soil had to be artificially infested with the smut. This was accomplished by grinding dried smutty onion seedlings and mixing this inoculum into the soil in the greenhouse bench. Also, a spore suspension was sprayed in the furrow immediately before seeding.

RESULTS

As mentioned above, the preliminary, June 1957, field trials were so seriously invaded by onion maggots that formal data were not taken. However, the plantings of HCB-treated onion seed were extensive enough to give a good indication of the efficacy of this fungicide in the control of onion smut. The preliminary results were highly encouraging. In two rows of onion seedlings approximately 100 feet long there was less than 1 percent of smut. The untreated checks showed considerable smut although it was not possible to determine the exact percentages because of onion maggot damage.

With this encouragement, a more elaborate series of seed treatments was set up for August 1957. Table 1 shows the fungicides and fungicide-insecticide combinations used. The seed was treated 2 to 4 weeks in advance of the planting dates, August 28 to 29. In addition to the seed treatments two soil fumigants were included, also indicated in Table 1. Data on the plots were taken the following mid-October.

The results of the August 1957 experiments were most encouraging with regard to the value of HCB for onion smut control. This fungicide was exceptionally good, especially in combination with certain insecticides used for the control of onion maggot. Taking into consideration percent reduction in smut, percent increase in stand, and average weight of the onion seedlings, three HCB-insecticide combinations were outstandingly successful: HCB plus Diazinon, HCB plus DDT, and HCB plus dieldrin.

Some of the other fungicides and fungicide-insecticide combinations gave some promise but did not measure up to HCB or the HCB-insecticide combinations. Thiram, for example, so widely used elsewhere for onion smut control, reduced smut only approximately 30 percent, although there was a 60 percent increase in stand.

The 1958 field experiments were conducted in the same manner as in 1957, with planting on September 3 to 4; the harvest was in mid-October, as before. Because of its outstanding promise in 1957, HCB figured prominently in the 1958 experiments. TCNA, because of its excellent control of smut (even though it did not contribute materially to stand increase) was also included. Although thiram gave little protection to onion seedlings against smut in the 1957 experiments, it was re-tested in 1958 principally because it has been used so successfully by Linn and Newhall (1) and others. Other fungicides added in the 1958 experiments were Dyrene, which Campbell[3] has used successfully to control onion smut under western Washington conditions, BSM-11, and Bayer 22555, as shown in Table 2.

Insecticides included in 1958 to keep onion maggots under control were Diazinon, DDT, endrin, and Guthion. These were applied in addition to, or in combination with, the fungicides. The test plot comprised three randomized blocks, containing 45 treatments replicated three times except where otherwise noted. Both HCB and TCNA were tested at the same rates as in 1957 and also at reduced dosage rates. The results of the 1958 field plantings are summarized in Table 2.

The outstanding and certainly unanticipated result from the 1958 experiments was the evidence of phytotoxicity resulting from the HCB treatments. In spite of the excellent smut control and generally good stands obtained with this fungicide in 1957, some pronounced instances of phytotoxicity resulted from the 1958 field experiments. As much as 4 weeks after planting, the HCB-treated seed had failed to germinate or had germinated only very slowly. In the meantime, other treatments, including the checks, had produced excellent seedling stands. The treatments, for example pelletizing, which permitted the HCB to remain on the seed during the germination process showed the most phytotoxicity. At reduced dosages, HCB showed a corresponding decrease in phytotoxicity.

It should be emphasized that the HCB used in 1958 was the same brand but a different lot from that used in 1957. Also, a different and evidently much more highly contaminated plot of ground was used for the 1958 experiments. This is evident when one compares the percentages of smut in the checks in the 2 years.

[3] Leo Campbell, Western Washington Experiment Station, Puyallup. Personal communication.

Table 3. Results of seed treatment with HCB (hexachlorobenzene) for onion smut control and its effects on seedling stands. Average of 2 replications of two 44-inch rows using seed pelletized with HCB at a 1:1 ratio by weight. Greenhouse tests conducted at Pullman, Washington.

Treatment	Number of seedlings	Percent smut	Size: Average weight per seedling (grams)
Anticarie 80[a]	311	8.6	.0964
Brand A 80	221	9.9	.0737
Anticarie 40	257	45.5	.1073
Brand B 40	244	47.9	.1040
"Homade"[b] 20	310	58.06	.1509
Brand C 40	179	63.1	.1055
Brand D 40	81	35.8	.0691
Brand E 40	42	9.5	.0523
Brand F 80	23	17.3	.0739
Brand G 40	37	18.9	.0405
Check	206	93.2	.0854

[a]The number indicates the percent of active ingredient.
[b]A laboratory formulation.

Table 4. Effects of a phytotoxic proprietary formulation of hexachlorobenzene on the germination rate of onion seed and subsequent seedling development. Seed germinated between moist paper toweling.

Treatment	Percent germination After 5 days	After 10 days	After 14 days	Approximate size (mm) after 20 days
Treated seed[a]	33-1/3	48	95	2.5-3.0 x 20.0
Untreated seed[b]	80	100	--	0.5-1.0 x 45.0-50.0

[a]Pelletized with 40 percent HCB plus 4 percent methyl cellulose sticker.
[b]Except for 4 percent methyl cellulose sticker.

Table 5. Efficacy of a single formulation of hexachlorobenzene[a] in the control of onion smut under greenhouse conditions. Pullman, Washington, 1958.

Treatment	Total seedlings recovered	Total smutty seedlings	Weight healthy seedlings (grams)	Average weight healthy seedlings (grams)	Percent smut
Treated seed[b]	3971	35	287.8	.0731	0.88
Untreated seed[c]	3640	2264	108.8	.0790	62.19

[a]"Anticarie 80" (see Table 3).
[b]Pelletized at the rate of 1:1 by weight, using 4 percent methyl cellulose sticker.
[c]Except for methyl cellulose sticker.

FIGURE 1. Severely smutted onion seedlings (7 weeks old) resulting from planting untreated onion seed in naturally infested soil. Infection severity is indicated by the numerous and extensive sori present causing a girdling of stems, leaves and cotyledons. Compare with Figures 2 and 4.

FIGURE 2. Lightly infected (few, small sori) but vigorous onion seedlings (7 weeks old) resulting from onion seed pelletized with a laboratory formulation of hexachlorobenzene containing 20 percent active ingredient. Seed planted in same soil as represented in Figure 1.

Though the 1958 results of the HCB treatments were disappointing and dismaying, a few of the other treatments showed more promise than they did in 1957. There were no impressive combinations of smut reduction and stand increase such as were obtained in the 1957 trials, but thiram in combination with DDT did result in a 58 percent reduction in smut and a 48 percent increase in stand. However, this 58 percent reduction in smut still meant that there was 40 percent of smut in the stands, and commercial practice requires better control than this. TCNA at reduced dosage and in combination with the insecticide Guthion gave a 77 percent reduction in smut and a 28 percent increase in stand. Here again, however, there was still more than 20 percent of smut in the stands. The high degree of soil infestation, as indicated by 92 percent of smut in the check rows, severely tested these fungicides in the 1958 experiments.

Because the same commercial brand of HCB gave excellent smut control and seedling stands in 1957, but extremely poor stands and only fair control of smut in 1958 (compare Tables 1 and 2), twenty 3-gram samples of onion seed were pelletized with nine different commercial formulations of HCB and one locally prepared formulation using technical HCB with a frianite diluent, in an attempt to reconcile these differences. The seed was pelletized

FIGURE 3. Upper row: Early effects of a phytotoxic formulation of hexachlorobenzene on the elongation of onion seedlings. Note the knobby root joints and thick, stubby seedlings. Lower Row: Normally elongated seedlings from untreated seed, same age as above.

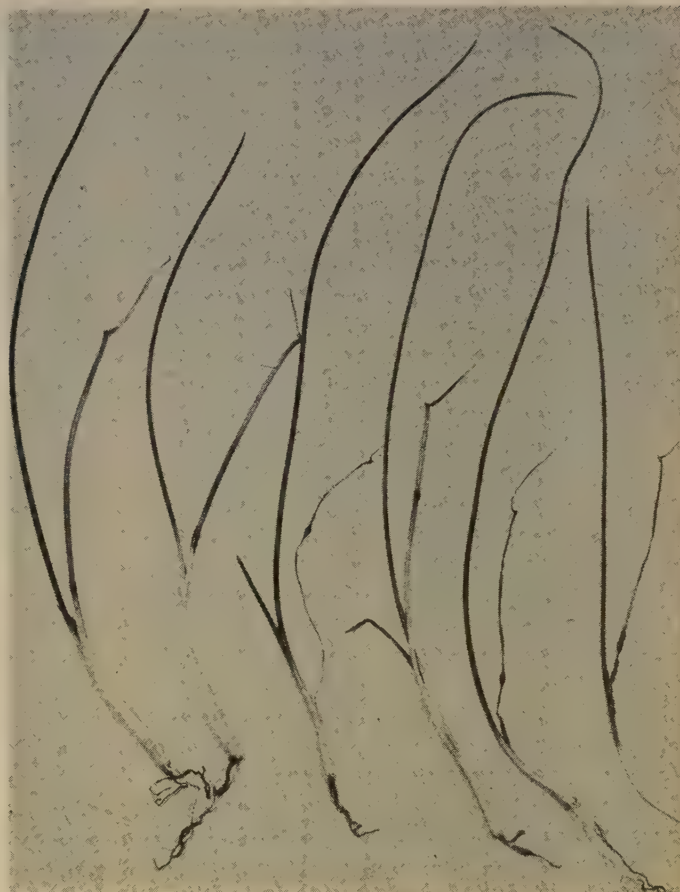

FIGURE 4. Smut sori, restricted to the cotyledons of onion seedlings, indicative of mild, non-systemic infections from which seedlings often survive. (Seed treated with "Anticarie 80").

as before, using a 4 percent methyl cellulose sticker. Two 3-gram samples treated only with the sticker served as checks. The experiment was set up in two randomized blocks involving 11 treatments and each treatment consisted of a 44-inch row replicated twice. The treated seed was sown December 13, 1958, in greenhouse benches containing soil infested 1 week earlier with pulverized smutty onion seedlings. Also, a spore suspension was sprayed in the furrows at the time of seeding. The resulting seedlings were harvested February 3 to 4, 1959, and data taken. The results are given in Table 3.

In taking smut counts of the 1958 field and greenhouse trials, it was noticed that the brands of HCB that were quite phytotoxic resulted in stubby seedlings, many of which remained permanently stunted. Phytotoxicity was measured by the reduction in number of seedlings and by their average weight as compared with the checks. The data in Table 3 indicate that again the fungicides were subjected to a rigorous test: there was 93 percent of smut in the untreated check rows. The data show considerable differences among the brands and strengths of the HCB formulations with respect to both phytotoxicity and smut control. The outstanding material in this test was the 80 percent HCB formulation known as "Anticarie." From the combined standpoint of stand, size of seedling, and degree of smut suppression, the 40 percent formulation

of this same proprietary product was not impressive. On the other hand another 80 percent HCB formulation, a different proprietary product, substantially reduced the amount of smut but also gave a poor stand.

It would appear from these data, then, that substantial differences in results can be expected from different formulations of HCB.

The onion seedlings growing in the greenhouse again showed that the various HCB formulations produced striking differences in smut control efficacy and in phytotoxicity. Eighteen days after planting, some of the HCB-treated seed lots had failed to germinate. Others, as well as the checks, had produced a heavy stand of seedlings. Differences in the severity of infection among treatments were also apparent. Figure 1 shows the severe infection in the checks. Lightly infected seedlings such as those in Figure 2 were growing vigorously when smut counts were made.

These greenhouse results thus substantiate the phytotoxicity results of the 1958 field trials as well as indicate the comparative efficacy of certain brands of HCB in controlling onion smut. Phytotoxicity was measured on the seedlings by comparing their numbers and their average size with the checks. This effect was also reproduced in the laboratory by germinating, between moist paper towels at room temperature, onion seed pelletized with one of the brands of HCB that had shown severe phytotoxicity. A similar but untreated (except for the methyl cellulose sticker) sample of onion seed served as a check. The effect of this particular brand of HCB on germination and the subsequent elongation of onion seedlings is shown in Table 4 as an over-all decrease in germination rate and obviously inhibited subsequent growth. The phytotoxic effect on the seedlings is illustrated in Figure 3.

To further test the safety and efficacy of "Anticarie 80," each of seven 3-gram lots of onion seed was pelletized with this material, again using a 4 percent methyl cellulose sticker. Seven 3-gram lots of untreated seed served as checks. The experiment consisted, thus, of two treatments replicated seven times. The treated and untreated seed were planted in greenhouse benches in rows approximately 44 inches long. The individual plots were laid out so that rows of treated seed always alternated with rows of untreated seed in all directions. Before sowing the seed in the rows the open furrows were sprayed with a suspension of viable onion smut spores, even though the soil in the benches had been artificially infested in connection with the previously described experiment. The first smut counts were made 33 days after planting and counts were continued sporadically for 10 days thereafter. As before, seedlings were scored as smutty regardless of the severity of the infection. However, it was obvious that the smutted seedlings in the untreated rows were generally severely diseased, whereas the smutted seedlings in the treated rows were only lightly infected, as indicated by the small and frequently inconspicuous sori.

Differences in vigor (based on average weights) between healthy seedlings produced by treated and untreated seed were not significant. Thus, even at high dosage rates, where seed was treated with an equal amount by weight of the fungicide, "Anticarie 80" had no detrimental effects on the seedlings and it conferred a very high degree of protection against smut (Fig. 4). The results of this test are summarized in Table 5.

CONCLUSIONS

The excellent control of onion smut obtained by treating onion seed with HCB indicates that this chemical holds great promise of replacing other fungicides in the control of this disease, particularly in regions where the other fungicides are not satisfactory. The results of the experiments clearly show a marked difference in the different formulations of HCB on the market. A few commercial brands of HCB are both efficacious in controlling onion smut and entirely non-phytotoxic. Others are less efficacious and highly phytotoxic. Our data indicate that these differences are due to some chemical variability among the various commercial preparations of HCB, as evidenced by differences in response to this fungicide by a single plant, the onion.

The poor germination of onion seeds and the subsequent suppression of their development caused by certain brands of HCB might be explained on the basis of auxin inhibition. Such inhibition is suggested in the laboratory tests in which onion seedlings from seed treated with certain HCB formulations failed to elongate normally, whereas the untreated seeds germinated rapidly and elongated normally.

At any rate, it is obvious that there are some serious and striking differences among the proprietary compounds on the market in which HCB is the active ingredient. Inasmuch as the percentage of this active ingredient does not explain the phytotoxicity, we must search for

other explanations. One possibility is the diluent used. Another is a difference in the technical grade of HCB used. It is conceivable that certain highly phytotoxic impurities are contained in some technical grades of HCB and not in others. These possibilities are being investigated.

In the meantime, until the cause of this pronounced phytotoxicity to onion seedlings is detected and eliminated, the only formulation of HCB that can be recommended for the control of onion smut is "Anticarie 80," and this recommendation must be made with the proviso that the manufacturers do not modify the ingredients of their formulation from that which has given such excellent results in the current experiments. This last note of caution is emphasized because, in the current experiments, some deviation in a single brand of 40 percent HCB must indeed have ensued from one year to the next. One lot of this commercial brand gave excellent smut control and showed no apparent phytotoxicity in 1957, but another lot made available in 1958 was disappointing in smut control and disastrous in phytotoxicity.

It is necessary, therefore, to detect and eliminate the exact cause of this phytotoxicity, because, aside from this factor, it has been demonstrated that HCB is an excellent and practical means of controlling onion smut.

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REDUCTION OF DECAY IN STORAGE GRAPES BY FIELD APPLICATIONS OF CAPTAN

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Summary

Gray mold rot (Botrytis cinerea), Cladosporium rot (Cladosporium herbarum), and Alternaria rot (Alternaria spp.) commonly develop in Emperor grapes stored at 32° F. Incidence of the various types is dependent on the harvest date and weather before harvest. Gray mold rot is predominant after rainfall while the other two types predominate during dry weather.

Fumigation with sulfur dioxide in storage is effective against spores on the surface of grapes, but does not control field infections established before harvest. Such infections have been reduced by applying captan in the vineyard. Three applications at monthly intervals before rainfall were effective in reducing decay. A single application after rainfall was not effective.

INTRODUCTION

The low temperature at which grapes are stored limits the number of molds or fungi that can attack them and cause decay. Storage at 32° F eliminates the development of black mold rot (Aspergillus spp.) and the common bread mold (Rhizopus spp.), which frequently occur in the vineyard. Refrigeration does not control certain other organisms that are capable of growth and development at the temperatures at which grapes are stored.

TYPES OF STORAGE DECAY

The principal types of decay that occur in storage are gray mold rot (Botrytis cinerea Pers. ex Fr.), Cladosporium rot (Cladosporium herbarum Lk. ex Fr.) and Alternaria rot (Alternaria spp.).

Gray mold rot is the most serious type of decay in storage and its various symptoms have led to several common names, such as "slip skin", "nest rot", and "bunch rot". Decay caused by B. cinerea is light brown. In the early stage of development the berry skin is loosened from the underlying tissues so that it slips under slight pressure. Later the whole berry is affected, but fumigation usually keeps the decay restricted to individual berries. Sometimes, however, decay spreads from one berry to another, producing the "nest rot" stage of decay which may be accompanied by growth of a gray mold over the surface of the berries.

Cladosporium rot is easily distinguished from gray mold rot. The color is black and the infections remain fairly firm and localized. Only rarely is the entire berry affected. Sometimes in the late stages of development the decayed portions of the berries are covered by a velvety-green growth of the mold.

Alternaria rot most commonly affects the berry near the capstem attachment, but may also occur at other positions. It causes a light brown, sunken decayed area that usually remains localized. Alternaria attacks the fibrous tissues (or "brush") at the capstem attachment, causing these to darken and weaken, permitting the berries to be shaken from the cluster.

RELATION OF DECAY TYPE TO WEATHER BEFORE HARVEST

The relative importance of each of these types of decay is related to the date on which a particular lot of fruit was harvested and the weather conditions that prevailed before harvest (Fig. 1).

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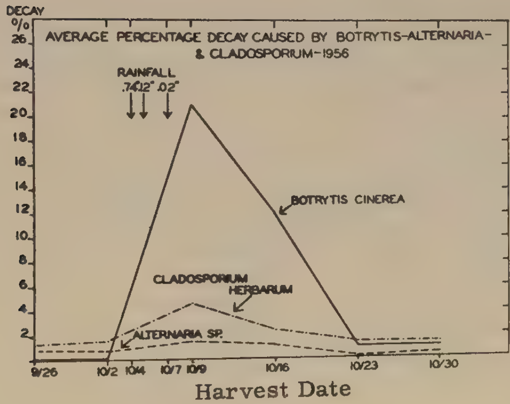

FIGURE 1. Percentages of various types of decay that developed after a 3-month storage period of Emperor grapes harvested at weekly intervals during the 1956 season.

When Emperor grapes were harvested at weekly intervals during the 1956 season and stored for approximately 3 months, it was found that early in the harvest season gray mold was not a problem and the decay that did occur was caused either by Cladosporium or Alternaria. After an early rain, however, gray mold became the predominant type of decay, the other two types being less affected by high moisture conditions. In 1956, the weather was relatively dry after this early rainfall. Under these conditions the percentage of gray mold rot gradually dropped off in the lots picked after the rain. By the third week after the rain the percentage had decreased almost to the level where it had been before the rain. This decline can be explained by the fact that decay in berries infected during the rainy period had developed far enough to be detected during packing and decayed berries were clipped from the clusters, leaving only relatively sound fruit to be placed in storage. The relative importance of the various decay types during the period following the rain would have been quite different had another rain occurred. In California rains are most frequent toward the end of the Emperor harvest and, as a general rule, the incidence of gray mold rot is also greatest then. In this respect 1956 was not a typical year.

THE EFFECT OF FUMIGATION ON DECAY

Fumigation with sulfur dioxide reduces the number of viable spores on the surface of grape berries, but does not control mold that has already gained entrance into the berries before harvest (2). Many of these preharvest infections have not developed far enough for the packers to cull them out and they cause serious decay in storage. Fumigation largely prevents new infections from occurring in storage. It also reduces the spread of decay from one berry to another, but it does not control infections that are already established. After berries infected with Botrytis cinerea have been fumigated with sulfur dioxide at concentrations as high as 5 percent for an hour, viable mycelium has been isolated from the inside of the berry. Since this type of infection could not be controlled by fumigation, the prevention of infection by applying some type of protective fungicide in the vineyards appeared feasible.

RELATION OF FIELD FUNGICIDES TO DECAY

Various fungicides have been tested for the control of gray mold rot of California grapes. One of the most effective of these materials has been captan (1). This fungicide can be applied as a dust, incorporating the regular sulfur dustings to avoid additional labor for application. The formulation tested contained 10 percent captan, 50 percent sulfur, and 40 percent inert carrier. It was applied at the rate of 20 pounds per acre. The duster was directed toward the grape clusters and was run through every row. The first application of dust was made before the clusters tightened up, that is, before the berries became enlarged enough to form a compact cluster. At this stage the dust could still penetrate into the bunch. The first application was made in the middle of July and was followed by additional dustings at 3- to 4-week intervals. The number of applications to individual plots is shown in Figure 2.

The percentage of decay at harvest was measured in a control and in the three different vineyard plots receiving captan treatments (Fig. 2). The fruit was picked 3 weeks after exposure to heavy rainfall (0.74 inches on October 4, 1956). Practically all the decay was gray mold.

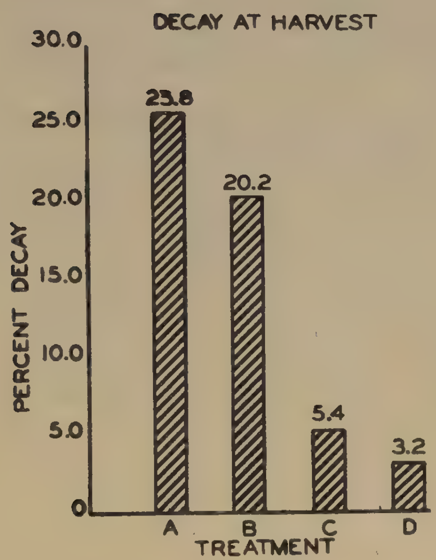

FIGURE 2. Percentage decay at harvest in Emperor grapes treated with captan: A -- Check; B -- One application the day following rainfall; C -- Three applications at monthly intervals before rainfall; D -- Three applications before rainfall and one the day following rainfall.

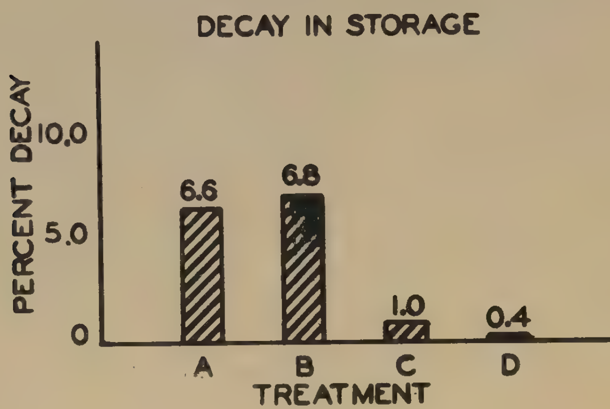

FIGURE 3. Percentage decay in Emperor grapes after 3-months' storage at 32° F: A -- Check; B -- One application of captan the day following rainfall; C -- Three applications of captan at monthly intervals before rainfall; D -- Three applications before rainfall and one the day following rainfall.

Decay was particularly severe in the check lot (25.8 percent). A single application of captan after the rain reduced decay by only 5.6 percent (to 20.2 percent). Three applications before the rain reduced decay to only 5.4 percent, and an additional application following the rain lowered the percentage of decay at harvest to 3.2 percent.

After measuring the decay at harvest, sound-appearing grapes from each of these lots were placed in storage. Grapes in which visible decay could be detected were discarded. These lots were held in storage for 3 months and received the customary fumigations with sulfur dioxide (3).

The percentage decay that developed during storage (Fig. 3) was about equal in the check (6.6 percent) and in the lot that received only one application of captan after the rain (6.8 percent). Only 1.0 percent decay developed in the lots that received three applications before the rain. Decay in storage was further reduced to 0.4 percent in the lot that had an additional application after the rain.

DISCUSSION AND CONCLUSIONS

Effective control of gray mold requires that the fungicide be applied before the fruit is exposed to rainfall. Applying the dust only after rainfall had little effect on infections that occurred during the rainy period, although it may have provided slight protection against secondary infections (that is, spread of decay from one berry to another).

Captan has been found to be effective against Botrytis, but previous work (1) has not shown that it significantly reduces decay caused by Cladosporium or Alternaria. Consequently, in dry years, when there is little Botrytis, and the small amount of decay that does occur is caused by these other organisms, there is little benefit from applying the fungicide.

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RELATIVE HUMIDITY IN CITRUS CARTONS AS INFLUENCED BY EXTERNAL TEMPERATURE AND RELATIVE HUMIDITY

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Summary

Effects of different external conditions of temperature and relative humidity on the relative humidity within fiberboard cartons of citrus fruits were investigated. When the temperature outside the carton was sufficiently lower than that of the contents to give a markedly lower vapor pressure than existed inside the carton, the internal relative humidity decreased rapidly. On the other hand, when the temperature and vapor pressure outside of the carton were sufficiently higher than those of the contents, the internal relative humidity increased rapidly. Moreover, in cartons of cold citrus the relative humidity increased to 100 percent and condensation developed on the fruit when the cartons were transferred to warm conditions in which the temperature of the fruit surface was below the dew point of the air outside of the cartons. In general, relative humidity changed more rapidly in vented cartons than in nonvented ones. Changes in relative humidity inside the carton resulted from movement of water vapor caused at least partly by a difference between the vapor pressure inside the carton and that outside of the carton. The water vapor moved in the direction of lower vapor pressure. Thermally-induced convection currents and moisture absorption by the carton walls also may have caused changes in relative humidity. After the initial drop in relative humidity when cartons of fruit were transferred to a lower temperature, there was a rise in relative humidity which would be sufficiently high for the release of ammonia from moisture-sensitive sheets used to control citrus blue and green molds.

INTRODUCTION

Ammonia-releasing sheets designed to control blue and green molds (*Penicillium* spp.) of citrus fruit are dependent on the absorption of moisture for the release of ammonia. These sheets are 11 X 17 inches and are made from absorbent paper toweling. One sheet is saturated with ammonium sulfate solution and then dried; the other is saturated with sodium carbonate solution and then dried (4). The two sheets are placed in contact when used in citrus cartons. One set of sheets contains an average of 1.025 grams of available ammonia. Because this formulation requires a relative humidity of about 85 percent to release ammonia, the relative humidity in cartons of citrus under different conditions of handling and storage was studied.

MATERIALS AND METHODS

To investigate the effect of external temperature and humidity on relative humidity within fiberboard containers, the relative humidity within cartons of lemons (size 150) and oranges (size 126) was measured before and after transfer of the cartons from one set of temperature and humidity conditions to different temperatures and humidities. Humidities were measured with an electric hygrometer consisting of a standard battery-type indicator and limited-range humidity-sensing elements. One set of these elements was placed in the middle of the carton and another at the end. The temperature of the air in the spaces between the packed fruits was measured by thermistors incorporated in the sensing elements. Vented as well as nonvented cartons of citrus were tested. The vented carton used in these tests had four slots (two in the top and two in the bottom), each approximately 1 X 4 inches.

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RESULTS AND DISCUSSION

When cartons of citrus were transferred to a space with temperature and vapor pressure considerably lower than those of the contents, relative humidity within the cartons decreased rapidly. For example, when a vented carton of lemons with internal air at 77° F and 90 percent relative humidity in the middle position was transferred to a room at 40° and 90 percent relative humidity, the relative humidity of the air inside of the carton dropped to 32 percent (Table 1; Fig. 1) as a result of a large vapor pressure differential between the air in the room and that in the carton. Similarly, when a vented carton of lemons with internal air of 60° and 88 percent relative humidity in the middle position was transferred to the 40° and 90 percent relative humidity room, the relative humidity inside the carton dropped to 62 percent (Table 1). At the higher temperature the vapor pressure inside the carton was 21.4 mm Hg, while at 60°F it was 11.7 mm, as compared with a vapor pressure in the precooling room air of only 5.7 mm. This difference seems to account for the greater drop in relative humidity with the greater temperature differential.

Nonvented cartons underwent smaller changes than vented cartons when transferred to the room at 40° F and 90 percent relative humidity. For example, in nonvented cartons of lemons with internal air at 77° F and 95 percent relative humidity, the internal relative humidity in the middle position dropped to 53 percent (Table 1 and Fig. 2), while in the vented cartons it dropped to 32 percent (Table 1 and Fig. 1).

Vapor pressure differences seem to account for the foregoing changes, where the transfer is from a higher to a lower vapor pressure.

When the transfer is from a higher to a lower temperature at about the same vapor pressure, there is also a lowering of relative humidity within the package. When cartons were equilibrated in a room at 77° F and 40 percent relative humidity and then transferred to one at 60° and 75 percent relative humidity, the transfer was not to a room with lower vapor pressure, but to one with slightly higher vapor pressure (10 mm Hg) than that (9.5 mm Hg) of the original room. The vapor pressure within the carton (21.4 mm Hg in the vented carton of lemons and 22.6 mm Hg in the nonvented one) was higher than that of either room, but the relative humidity dropped only when the carton was transferred to the room with lower temperature. In the vented carton the relative humidity dropped from 90 to 60 percent (Table 1 and Fig. 3), and in the nonvented one from 95 to 70 percent (Table 1 and Fig. 4).

Possibly water vapor is swept out of the carton by continuous convection currents when there is a change in temperature but not in vapor pressure in the air surrounding the package. Convection currents could pull water vapor out of even a nonvented carton, in spite of the resistance offered by the fiberboard material to mass movement of air, because of air leaks in various places, for example, around the glued joints of the top and bottom, and where the telescopic lid fits over the bottom half of the carton.

Although moisture did not condense on it the carton wall probably absorbed some of the water vapor that left the interior of the carton, as shown by softening and weight gain of the fiberboard during cooling of the fruit. This absorption may have a part in the reduction of relative humidity within the carton. The relative importance of vapor-pressure difference, convection currents, and absorption by the carton wall has not been determined.

Table 1 summarizes changes in relative humidity in cartons of citrus following transfer to lower temperature. Comparison of humidity in vented and nonvented cartons of oranges and lemons showed that differences in relative humidity due to type of fruit are insignificant.

When cartons of citrus were transferred to spaces with temperatures and vapor pressures considerably higher than those of the contents, the relative humidity within the cartons increased markedly. For example, when freshly packed, nonvented cartons of lemons at 60° F were transferred to 85° and 55 percent relative humidity and 77° and 63 percent relative humidity, the relative humidity of the internal air in the middle position increased and reached saturation with resultant condensation in about 30 minutes at 85°, and in about 45 minutes at 77° (Fig. 5). In vented cartons of the fruit at 60° treated in the same manner condensation occurred immediately.

The marked rise in relative humidity in nonvented cartons transferred to higher temperatures is probably due primarily to the movement of water vapor, from air at the higher vapor pressure outside, to air at the lower vapor pressure inside, the carton. There could be only a very slight increase in transpiration rate of the fruit, since its temperature rose very slowly after transfer (Fig. 5). Vapor pressure in a nonvented carton of lemons at 60° F and 90 percent relative humidity was 12 mm Hg. When this carton was transferred to a room at 85° F and 55 percent relative humidity, water vapor migrated into the carton because of the higher

Table 1. Relative humidities in vented and nonvented cartons of citrus as affected by transfer to lower temperatures.

Change of external conditions	Kind of fruit	Type of carton	Drop in percent relative humidity in middle position From	To	Drop in vapor pressure (mm) in middle position From	To	Number hours for relative humidity to rise to 85%
From 77° F and 40%	Lemons	Vented	90	32	21.4	7.3	26
RH (9.5 mm VP)		Nonvented	95	53	22.6	11.8	29
to 40° and 90% RH	Oranges	Vented	88	31	20.9	7.1	26
(5.7 mm VP)		Nonvented	93	50	22.1	11.1	29
From 60° F and 75%	Lemons	Vented	88	62	11.7	7.6	20
RH (10.0 mm VP)		Nonvented	92	73	12.2	9.0	20
to 40° and 90% RH	Oranges	Vented	85	61	11.3	7.4	20
(5.7 mm VP)		Nonvented	90	72	12.0	8.9	20
From 77° F and 40%	Lemons	Vented	90	60	21.4	13.6	30
RH (9.5 mm VP)		Nonvented	95	70	22.6	16.0	25
to 60° and 75% RH	Oranges	Vented	88	59	20.9	13.5	30
(10.0 mm VP)		Nonvented	93	68	22.1	15.6	25

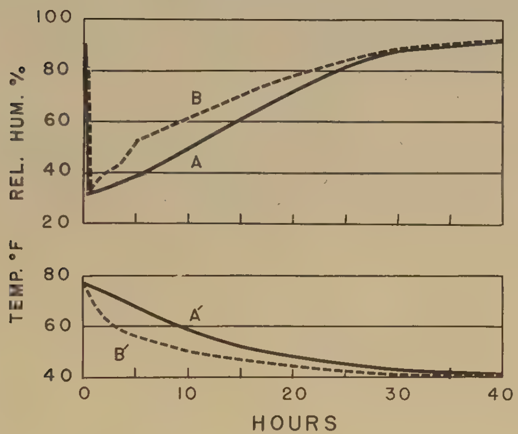

FIGURE 1. Relative humidity in vented carton of lemons as affected by transfer to lower temperature. Carton equilibrated in a 77° F room with 40 percent relative humidity, then transferred to room at 40° and 90 percent relative humidity. A -- Relative humidity in middle position; B -- In end position. A', B' -- Temperatures in the respective positions.

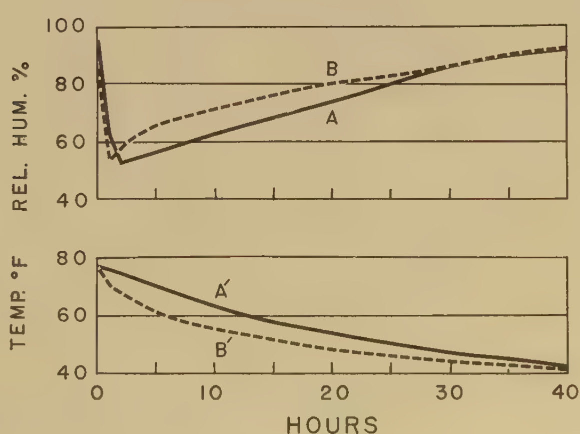

FIGURE 2. Relative humidity in nonvented carton of lemons as affected by transfer to lower temperature. Carton equilibrated in a 77° F room with 40 percent relative humidity, then transferred to room at 40° and 90 percent relative humidity. A -- Relative humidity in middle position; B -- In end position. A', B' -- Temperatures in the respective positions.

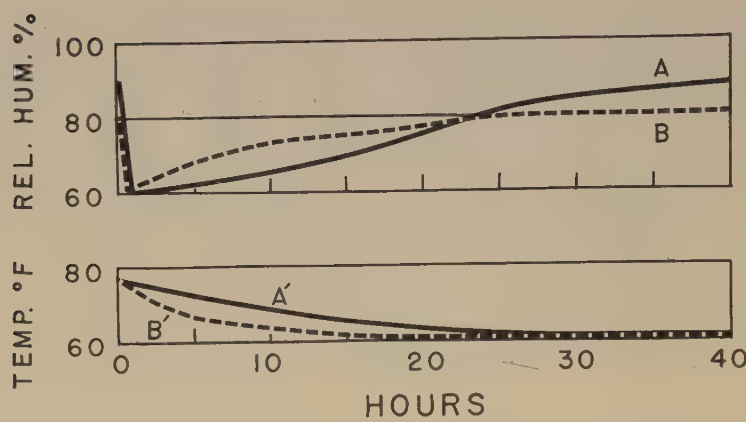

FIGURE 3. Relative humidity in vented carton of lemons as affected by transfer to lower temperature. Carton equilibrated in a 77° F room with 40 percent relative humidity, then transferred to room at 60° and 75 percent relative humidity. A -- Relative humidity in middle position; B -- In end position. A', B' -- Temperatures in the respective positions.

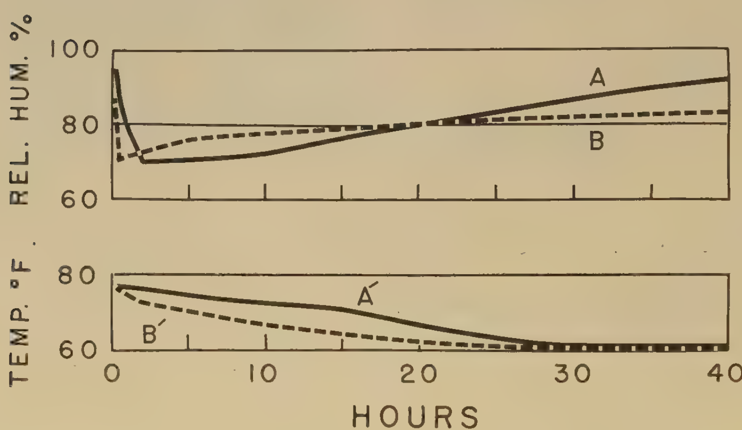

FIGURE 4. Relative humidity in nonvented carton of lemons as affected by transfer to lower temperature. Carton equilibrated in a 77° F room with 40 percent relative humidity, then transferred to room at 60° and 75 percent relative humidity. A -- Relative humidity in middle position; B -- In end position. A', B' -- Temperatures in the respective positions.

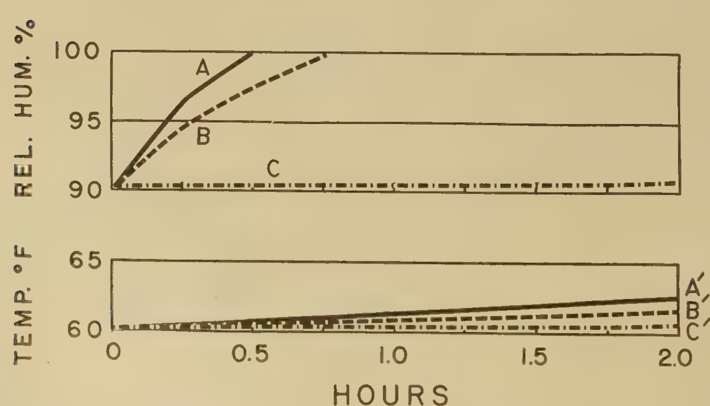

FIGURE 5. Relative humidity in middle position in freshly packed nonvented carton of lemons at 60° F transferred from 65° air with 56 percent relative humidity to A -- 85° air with 55 percent relative humidity, and B -- 77° air with 63 percent relative humidity. C -- Relative humidity in carton left in room with 65° temperature and 56 percent relative humidity. A', B', C' -- Temperatures in the respective positions.

vapor pressure (16.9 mm Hg) in the surrounding air. Condensation occurred because the fruit temperature of 60° was below the dew point (66°) for the given air temperature and relative humidity. Likewise, when a similar carton was transferred to a room at 77° and 63 percent relative humidity, humidity within the carton increased because of the higher external vapor pressure (14 mm Hg), and condensation occurred because the fruit temperature of 60° was below the dew point (64°). In a carton left standing in a room at 65° and 56 percent relative humidity moisture did not build up because the lower vapor pressure, 8.8 mm Hg, in the outside air enabled water vapor to move out of the carton, and condensation did not take place because the fruit temperature of 60° was above the dew point of the outside air (48.5°).

The results of this work are related to those obtained by Curtis (1), who used vapor-pressure difference as a basis for explaining the migration of moisture in apples. In his experiment, apples were exposed to warm air on one side and to refrigerated air on the other. Shriveling developed on the warm side whereas the cool side remained turgid. Since one side of each apple was insulated from the opposite side, Curtis attributed the difference in turgidity to the movement of water vapor through the intercellular spaces of the parenchymatous tissue from the higher vapor pressure of the warm side to the lower vapor pressure of the cool side.

In accord with the principle brought out by Curtis' work (1), it was found that increase or decrease in relative humidity inside the carton is the result of inward or outward movement of water vapor due, at least partly, to difference in vapor pressure between the spaces inside and outside of the carton, the movement of water vapor being in the direction of lower vapor pressure.

The general principle behind the behavior of relative humidity also is borne out by the work of Heaton et al. (2), with cartons of canned goods moved from refrigerated to nonrefrigerated conditions and vice versa.

According to Roistacher et al. (3), ammonia is effective against blue and green molds (Penicillium spp.), if applied within 24 to 30 hours after inoculation with spores when the average temperature of the fruit is 68° F, about 40 hours at 60°, and about 80 hours at 50°. Even though the relative humidity within cartons of citrus transferred to 40° drops below 85 percent (the relative humidity needed to release ammonia from the sheets), it does not remain below 85 percent for longer than 29 to 30 hours (Figs. 1 and 2). At an average temperature of 60° or below in the middle position (Figs. 1 and 2), the time at which the humidity would be high enough for the ammonia to be released is within the 40-hour period after inoculation with spores. However, if the fruit were infected before packing, the effectiveness of ammonia sheets might be reduced by the delay in time required for the humidity in the package atmosphere to be built back up.

In both vented (Fig. 1) and nonvented cartons (Fig. 2), the relative humidity recovered more rapidly in the end positions than in the middle positions, because the fruit cooled more rapidly in the end positions.

Upon removal to warm air, ammonia is released rapidly from sheets in contact with condensate in cartons of cold fruit. The effect of condensate on the ability of ammonia to control decay has not been fully investigated.

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TREATMENT OF SOYBEAN SEED IN MINNESOTA[1]

T. D. Wyllie[2] and R. W. Goth[3]

The effect of treating experimental lots of soybean seeds with fungicides has been studied in Minnesota (7) and at other locations (1, 2, 3, 4, 5, 6, 8, 9, 10); in general, such treatment resulted in emergence of more seedlings and survival of more plants. There has been no information available, however, on the response of commercial seed lots to fungicidal seed treatment in Minnesota, and the present studies were undertaken to determine this.

MATERIALS AND METHODS

Seed lots -- Commercial seed lots were obtained from farmers in 32 counties in Minnesota[4], and certified lots of the varieties Renville, Blackhawk, and Chippewa were obtained from the State Seed Laboratory stocks.

Test procedures -- Half of each sample was treated with thiram (tetramethylthiuram disulfide) at the recommended rate of 2 ounces/bushel, and the other half was not treated. Each of the portions was then divided into three samples of 200 seeds each, and these were planted in three rows, each row 18 feet long, the treated and nontreated seed in adjacent rows. In 1956, plants were not counted, but in 1957 and 1958 plants were counted 14 days after planting.

In 1958 thiram was applied at 20 times the normal rate in one test (to determine possible phytotoxic effects of an excessive dosage) and the seeds were planted at depths of 1 inch and 3 inches. The plot was irrigated daily with overhead sprinklers from the time of planting until the seedlings emerged. A split-plot design was used.

RESULTS

Stands from nontreated seed planted at a depth of 1 inch were about 30 percent greater than stands from those planted 3 inches deep (Table 1). Stand and yield from the treated seed planted 3 inches deep were virtually the same as those from nontreated seed planted at a depth of 1 inch. Poor stands and significant decreases in yield resulted when nontreated seed of the varieties Comet and Grant were planted 3 inches deep; however, Comet was affected more than Grant. These data indicate that Grant is able to produce a fairly good crop even if large stand decreases occur.

During 1957 and 1958 the effect of seed treatment on 68 commercial soybean seed lots of Renville, Blackhawk, and Chippewa was studied in the field. The stand was increased in 74 percent of the samples (Table 2).

Fair-quality seed (stand counts of nontreated samples were used as the criterion of quality) responds more to chemical seed treatment than seed of higher quality (Figure 1). Response to treatment was measured in terms of increases in stand following treatment. The differences in stand illustrated in Figure 1 are not statistically significant, but a trend is evident. The results of these tests are based primarily on the response to seed treatment of fair- to good- quality seed lots (stands of 75 percent or more). Since only a few seed lots of poor quality were tested, the effect of treatment on seed of poor quality is uncertain.

Seed treatment increased yields in an average of 75, 65, and 43 percent of the seed lots tested in 1956, 1957, and 1958, respectively (Table 2). The 1958 growing season was exceptionally dry and generally unfavorable for disease development at St. Paul. This may explain

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[1]Paper No. 4168, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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[4]The authors express their appreciation to Mr. John Larson of the Minnesota State Seed Testing Laboratory, University of Minnesota, St. Paul, for his cooperation in making these samples available.

Table 1. Effect of seed treatment and two depths of planting on stand and yield of two varieties of soybeans at St. Paul, Minnesota.

Variety and treatment	Planting depth (inches)	Stand[a] (numbers)	Stand reduction (per cent)	Yield[b] (grams)
Comet:				
Thiram	1	169	16	1254
	3	136	32	1110
None	1	129	36	1122
	3	87**	66	878*
Grant:				
Thiram	1	180	10	1350
	3	142	29	1423
None	1	143	29	1344
	3	76**	62	1128*

[a]200 seeds per replication; average of 3 replications.
[b]Average of 3 replications.
*Significant at the 5% level.
**Significant at the 1% level.

Table 2. Effect of seed treatment on stand and yield of soybean varieties at St. Paul, Minnesota.

Year	Variety	No. samples tested	Percentage samples[a] showing stand increase	Percentage samples[b] showing yield increase
1956	Renville	9	--	67
1956	Blackhawk	11	--	82
1957	Renville	13	77	62
1957	Blackhawk	15	73	67
1958	Blackhawk	20	75	40
1958	Chippewa	20	70	45
Total		88	Average 74	Average 61

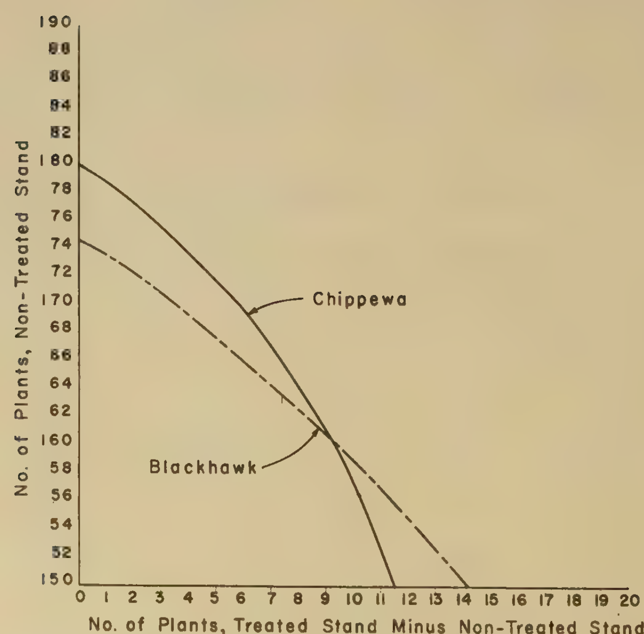
[a]Based on 400 seeds planted per plot, average of 3 replications.
[b]Each test based on a total of 400 seeds planted per replication, average of 3 replications.

Table 3. Range of response in yield of three varieties of soybeans in three years through seed treatment.

			Yield (bu/a)[a]					
		No. samples	Reduction			Increase		
Year	Variety	tested	No.	Range	Ave.	No.	Range	Ave.
1956	Renville	9	3	0.9-5.4	3.0	6	0.0-3.6	1.4
1956	Blackhawk	11	2	0.2-2.9	1.5	9	1.5-7.2	3.1
1957	Renville	13	5	0.6-6.3	3.1	8	1.0-4.3	2.3
1957	Blackhawk	15	5	0.8-4.7	2.8	10	1.0-8.1	2.5
1958	Blackhawk	20	12	0.2-8.2	1.8	8	0.0-5.9	2.3
1958	Chippewa	20	11	0.1-4.4	1.4	9	0.0-2.2	1.1

[a]Based on a total of 400 seed planted per plot per sample, replicated 2 times, for each variety in each year.

Figure 1. THE EFFECT OF SEED QUALITY ON STAND RESPONSE OF TWO VARIETIES OF SOYBEANS TO SEED TREATMENT IN 1958[1]



1- Curves based on a quadratic analysis of the data.

FIGURE 1

why an average of only 43 percent of the samples showed an increase in yield as compared with the higher percentages obtained in 1956 and 1957.

Although stand was increased in 74 percent of the samples and yield in 61 percent of the samples tested, the most important consideration is the amount of increase in yield that can be expected within a given seed lot. Table 3 shows the range and the average for yield in each of the test years.

During 1956 through 1958 a total of 22 percent of the treated samples had yield increases in excess of 2 bushels/acre and 53 percent had yield increases in excess of 1 bushel/acre. The maximum increases in yield obtained in 1956, 1957, and 1958 were 7.2, 8.1, and 5.9 bushels/acre, respectively, which are highly significant. Of the 87 samples tested, 11 had an increase of 2.6 or more bushels per acre (significant at the 5 percent level) and 11 had significant reductions. The overall average increase in yield of all seed lots tested, however, was not statistically significant because of the large amount of variation in the test seed lots. A trend in favor of seed treatment is indicated however, by an increase in yield in 49 of the 87 seed lots tested.

DISCUSSION

The value of soybean seed treatment apparently depends upon several factors: 1) the quality of the seed, 2) weather and soil conditions immediately following planting and while plants are in the seedling stage, and 3) the depth of planting. The studies demonstrate that, in general, fair-quality seed responds more to seed treatment than does high-quality seed, because of the general lack of vigor in poorer quality seed and because of the cracks and abrasions in the seed coat that may afford avenues of entry for root-rotting and seed-decaying organisms. This is particularly true when soil conditions are unfavorable for germination and the seed lies in the ground for prolonged periods. Although the majority of the commercial seed lots responded favorably to treatment, many did not respond and large yield reductions occurred in some cases. This variability of response has been reported by other investigators (3) who have made similar studies. Why certain seed lots respond to seed treatment and others do not is not known. Until further studies are made and the reasons for such tremendous variations are elucidated, seed treatment of soybeans cannot be recommended as a general practice; nevertheless evidence indicates that treatment may protect the seed against detrimental effects resulting from deep planting and other unfavorable conditions affecting germination and emergence.

One approach to a solution to the complex problem might be to increase the size of test plots and the number of replications in an attempt to minimize the apparently inherent variability in studies of this type.

No evidence of injury caused by thiram to soybean was found in this study, at either the recommended application rate of 2 ounces/bushel or at excessive dosages, nor was evidence found to suggest that seed treatment adversely affected nodulation. Apparently adequate populations of nodule-forming bacteria were present in the soil, since soybeans had been grown on the land in previous years.

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THE EFFICACY OF PREPLANT AND POSTPLANT APPLICATIONS OF 1,2-DIBROMO-3-CHLOROPROPANE FOR CONTROL OF THE STING NEMATODE, BELONOLAIMUS LONGICAUDATUS

W. E. Cooper, J. C. Wells, J. N. Sasser, and T. G. Bowery

Abstract

The effectiveness of 1,2-dibromo-3-chloropropane 17.3 percent granular, as a control of the sting nematode, was evaluated at four rates, 0.0, 0.5, 1.0, and 1.5 gallon per acre of technical fumigant, in preplant applications on peanuts, cotton, corn, and soybeans, and in postplant applications on peanuts, corn, and soybeans. The most effective preplant treatments increased yields approximately 500, 400, and 100 percent for soybeans, peanuts, and corn respectively. Early postplant treatments (1 month after planting) of peanuts and soybeans increased yields at the 1.0 and 1.5 gallon rates. Total bromide content of the shelled peanuts and peanut hay was analyzed.

INTRODUCTION

The sting nematode, Belonolaimus longicaudatus Rau 1958 (6)[1], is perhaps the most devastating nematode parasite of peanuts and appears to be widespread in the peanut belts of Virginia, North Carolina, and South Carolina (3). Its known distribution in North Carolina comprises 16 counties with 8 of these being major peanut producing counties.

Control of this nematode by crop rotation is extremely difficult since all of the major crops grown in rotation with peanuts (corn, soybeans, cotton) are also highly susceptible to the sting nematode. The growing of these crops in rotation with peanuts in the past has undoubtedly contributed to the present severity of the nematode problem in these areas.

Various soil fumigants have reduced the population levels of the sting nematode to a point sufficient for good crop growth (2, 4, 5, 8). The present investigations were carried out to determine: 1) effective rates and relative effectiveness of preplant and postplant applications of Nemagon[2] on nematode control and yield and quality of peanuts, corn, soybeans, and cotton, and 2) to determine if toxic residues were present in the harvested peanut crop. A preliminary report has been given (1).

MATERIALS AND METHODS

Location and Soil -- The field selected for this experiment was located on the Barkeley Estate about 1 mile N.N.E. of Severn, North Carolina. It had a history of very poor yields of all crops grown for several years. Soybeans the preceding year were almost a complete failure. The soil type was Norfolk loamy fine sand. The soil throughout the test area was relatively uniform, and, judging from performance of the preceding soybean crop and the results of the soil sample assay, the entire area was heavily infested with the sting nematode. No other parasitic forms occurred in any appreciable number.

Soil Preparation -- The debris of the preceding soybean crop was buried by turning the soil in March. The field was disced and harrowed just prior to application of the preplant treatments.

Treatments and Plot Design -- The nematocide treatments consisted of four rates: 0.0, 0.5, 1.0, and 1.5 gallons per acre of dibromochloropropane (DBCP) applied as a 17.3 percent granulated formulation on each of three dates: one preplant (2 weeks prior to planting) and two

[1] For many years, the sting nematode on various crops in the southeast has been referred to as Belonolaimus gracilis Steiner, 1949, this being the only species then described for this genus. In a recent paper Rau described another species which he named B. longicaudatus. Specimens collected from the field where these tests were conducted have been identified by Rau as B. longicaudatus.

[2] Nemagon soil fumigant, 1,2-dibromo-3-chloropropane (DBCP) was used in this test and was supplied through the courtesy of Shell Chemical Corporation.

postplant dates (June 9 and July 11). In preplant treatments, the DBCP was applied approximately 6 inches deep in the row with an accurately calibrated, modified Gandy distributor. In postplant treatments it was applied carefully by hand to open furrows 4 to 5 inches deep and approximately 6 inches to either side of each 25-foot row.

The experimental design was four replicates of a randomized block with split plots. The whole plots represented date of treatment and the split plots the rate of application. In addition, rows of each of the four crops, peanuts, soybean, corn, and cotton, were planted in each split plot. Each crop was planted in a block; however, its position within the replicate was at random.

Determination of the Nematode Population -- Soil samples were collected with a 1-inch diameter sampling tube from the top 6 to 8 inches of the root zone of representative plots on June 9, August 11, and October 28. Nematodes were recovered from 1 pint of soil by a combination of screens and modified Baermann funnel techniques, and counted with the aid of a dissecting microscope (36X).

Fertilization and Pest Control -- For the most part each crop was fertilized and treated with pesticides according to specific recommendations of the North Carolina Agricultural Extension Service.

Varieties and Planting -- The following varieties were used in these tests since they are considered to be well adapted to this area: Coker 100 W cotton, NC 27 corn, NC 2 peanuts, and Lee soybean. All crops were planted with hand equipment on May 2, 1958, in rows freshly marked off without disturbing the soil below the level at which the seed were placed. A very heavy rain and low temperatures immediately followed planting. Presumably, these unfavorable conditions resulted in very poor stands of peanuts and cotton and some skips in the corn and soybeans. The cotton plots and missing hills of the other crops were replanted May 14. Following this replanting, relatively uniform stands of all four crops were obtained.

Harvest Operations -- All crops were harvested on October 28. The soybean plants from each plot were cut, tied into a bundle, and stored until thoroughly dry. They were then thrashed with a small-plot soybean thrasher and the yields determined.

Peanuts were dug with a tractor-mounted 2-row digger-shaker. The plants from individual plots were stacked on a small stack pole until dry, December 2, at which time they were picked with a carding type Victory picker. The varying amounts of broken vines that came from the picker with the peanut pods were removed by hand. After individual plot yields were determined, the pods from the four replicates of each treatment were bulked and thoroughly mixed, and two kilogram samples were drawn, one for chemical analysis and one for grade analysis. Just prior to picking, a sample of the peanut hay (foliage and stems) was taken from the plants from each plot and like treatments were pooled for chemical analysis. The method used for the total bromide analysis was that of Shrader (7), as modified by R. W. Young, Department of Biochemistry, Virginia Polytechnic Institute, Blacksburg, Virginia.

The corn was husked in the field. The number and total weight of ears from each plot were recorded.

Owing to obviously severe yield reductions by boll worm and boll weevil, which were independent of the treatments under study, it was considered desirable to estimate the cotton yield by counting the total number of bolls produced per plot and multiplying this by a common factor.

EXPERIMENTAL RESULTS

Growth Response

Differences in the development of plants in the preplant plots appeared very early in all crops. In cotton, so many of the plants in the non-preplant treated plots had died by the time for the first postplant treatment, June 9, that the postplant treatments were not applied. By mid-July, nearly 100 percent of the cotton plants were dead in all plots which had not received preplant treatment with the nematocides.

Early responses of corn, soybean, and peanut to preplant fumigation were shown by an increase in plant size and a darker green color. With the exception of corn, these differences became greater as the season progressed.

Growth response of peanuts and soybeans to the early postplant treatments was evident by early August. However, it was not nearly so great as in the preplant treatments. Response to the second postplant treatments was much less evident throughout the season, although in some cases the plants in the treated plots appeared to be larger than those in the corresponding nontreated plot.

Yield Response

Peanuts and soybeans gave a similar response, with increased yields of approximately 400 and 500 percent, respectively, for the higher rate of the preplant treatment (Fig. 1). The lower rates were less effective in increasing yields. The earlier, June 6, postplant treatments gave similar, though less pronounced, yield increases. The last, July 11, postplant treatments were relatively ineffective.

In general, corn gave the lowest yield increase with nematocide treatments (Fig. 1). There was, however, a 100 percent increase from the 1.0 gallon per acre preplant treatment. Postplant treatments did not significantly increase corn yields.

Because of stand failure in all cotton plots which did not receive a preplant treatment with a nematocide, the determination of the response of cotton was limited to the preplant treatments. In these the 1.0 and 1.5 gallon per acre rates gave estimated yields of approximately twice as much as the 0.5 gallon rate (Fig. 1). Fusarium wilt (*Fusarium vasinfectum* Atk.) (= *F. oxysporum* f. *vasinfectum* Snyd. & Hans.) was very severe at the lowest rate and less severe at the higher rates of the nematocide. Late planting and insect damage were factors contributing to the low yields.

Effect on Quality

Peanut grades as indicated by the percentage of sound mature kernels was directly correlated with the increased yields (Table 1). Values per acre calculated from support price, which is based upon grades, show even greater differences between treatments. This is especially true for those treatments which did not produce peanuts that qualified for support price for edible trade.

The average ear weight of corn was directly correlated with yield (Table 2). Preplant treatment resulted in significantly larger ears. The trend for reduced ear weight at the higher dosages may indicate phytotoxicity.

Nematode Control

Populations of the sting nematode on peanuts as influenced by various dosage levels of DBCP applied as a preplant treatment are shown in Figure 2. Populations were reduced greatly in all treated plots as shown by the numbers present on June 9, approximately 2 months after treating. Population increased most during the growing season in those plots treated at the 0.5 gallon rate, intermediately at the 1.0 gallon rate, and least at the 1.5 gallon rate. Populations in the untreated controls did not change appreciably during the growing season.

In general, the effect of the postplant application of DBCP on the population level was about the same as the preplant treatment (Fig. 3). That is, the populations 2 months after treatment were lower in all treated plots; however, with the exception of the 1.5 gallon per acre rate the populations subsequently increased to higher population levels than in the nontreated plots. The degree of control was directly proportional to the rate used.

Bromide Residue

Total bromide residues found in shelled peanuts from plots treated with DBCP at 0.5 and 1.0 gallon per acre were no higher than in the untreated controls (Table 3). At 1.5 gallon per acre, the total bromides were detected in quantities considerably higher than in the controls. The date of treatment did not appear to influence the bromide residue in shelled peanuts.

Peanut hay analysis showed a much higher total bromide content which was directly proportional to the dosage applied. Side-dress applications in general resulted in lower bromide uptake than preplant treatments.

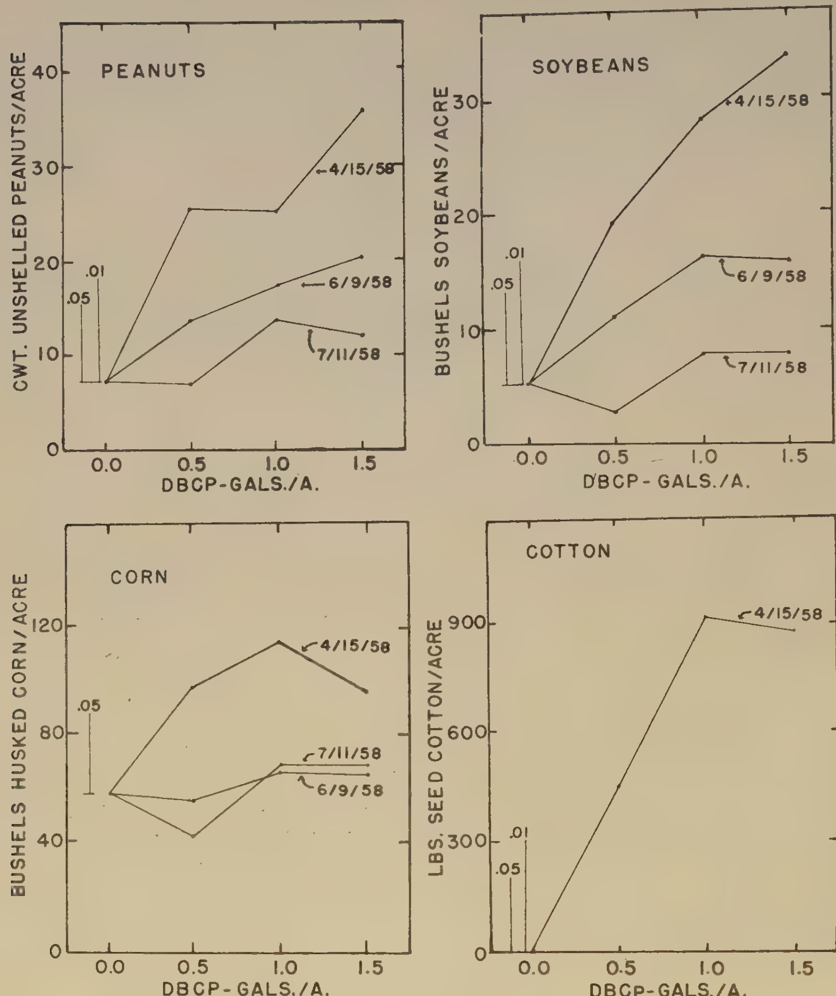

FIGURE 1. The effect of rate and date of DBCP fumigation of sting nematode infested soil on peanut, soybean, corn, and cotton yields. With the exception of cotton the indicated L. S. D. values are for rates within date interaction.

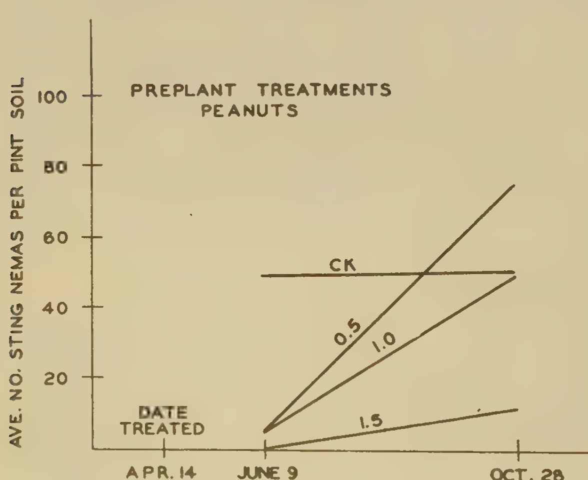

FIGURE 2. Sting nematode populations on peanuts as influenced by different rates of DBCP applied as preplant treatments.

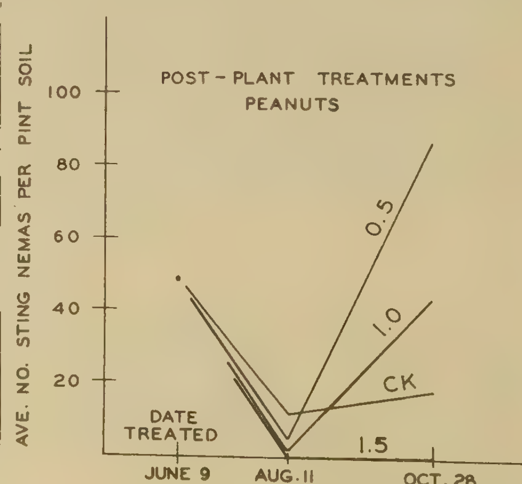

FIGURE 3. Sting nematode populations on peanuts as influenced by different rates of DBCP applied as postplant treatments.

Table 1. Quality, market price, and value per acre of peanuts as influenced by rate and time of application of DBCP.

Treatment		Quality evaluation				Support	
		Percent	Percent indicated kernels/100 gm. pods			price/100 lb.	Calculated
Date	Rate (gal./A.)	fancy sized pods	SMK[a]	DK[a]	OK[a]	unshelled nuts	value/acre
Check	0.0	15	58	11	6	$6.00[b]	$43.94
Preplant (4/15/58)	0.5	38	66	7	3	9.65	248.10
	1.0	41	72	4	1	11.49	289.89
	1.5	46	71	4	2	11.43	404.16
Postplant (6/9/58)	0.5	34	62	10	3	6.00[b]	82.62
	1.0	38	69	3	4	11.31	196.91
	1.5	43	72	3	1	11.66	236.81
Postplant (7/11/58)	0.5	25	61	10	4	6.00[b]	42.24
	1.0	34	66	7	2	9.44	129.80
	1.5	50	69	2	4	11.42	137.38

[a]SMK=sound mature kernels; DK=damaged kernels; OK=other kernels.
[b]Quality too low to qualify for support price; valued at $6.00/100 lb. for oil stock.

Table 2. The influence of rate and date of DBCP treatment on corn ear size.

Rate of fumigant (gal./A.)	Ear weight (ounces) By date of treatment: Check	4/15	6/9	7/11	Average ear weight
0.0	5.4	-	-	-	5.4
0.5	-	5.9	5.6	4.4	5.3
1.0	-	6.3	5.8	5.7	5.9
1.5	-	6.0	5.0	5.0	5.4
Average	5.4	6.1	5.4	5.1	

L. S. D. between dates .05 = .442

Table 3. Total bromide content of shelled peanuts and peanut hay from plots treated with DBCP.

Treatment: Date	Rate (gal./A.)	Total bromide (ppm)[a]: Shelled peanuts	Peanut hay
Preplant (4/15/58)	0.5	0	338
	1.0	0	681
	1.5	39	821
Postplant (6/9/58)	0.5	0	81
	1.0	0	174
	1.5	16	299
Postplant (7/11/58)	0.5	0	120
	1.0	0	276
	1.5	31	478
Untreated check		8	98

[a]Each value corrected for check.

DISCUSSION AND CONCLUSIONS

Data herein presented indicate that sting nematode injury can result in severe losses to peanuts, corn, cotton, and soybeans. Preplant treatment at the most effective rates increased yields approximately 500, 400, and 100 percent for soybeans, peanuts, and corn, respectively. Early response of the various crops to preplant treatments is indicative of early activity of the nematode. Although considerable yield increases resulted from postplant applications of DBCP to corn, soybeans, and peanuts, it would appear that preplant treatments should be used in fields known to be infested with the sting nematode. Because of early season stand failure of cotton, apparently associated with nematode damage, only preplant treatments should be applied. In fields where the presence of the nematode is detected after planting of corn, soybeans, or peanuts, side-dress applications may be effective but should be applied as soon as possible after detection of the nematode disease.

In general, nematode control was correlated with crop performance. The large late season increase in the nematode populations in plots treated at the 0.5 and 1.0 gallon per acre rate was probably due to the reduction in numbers of nematodes at the beginning of the growing season, thus enabling the plant to develop a good root system. This, in turn, enabled the surviving nematodes to increase rapidly, therefore populations were higher at the end of the season in treated plots. The crops, however, were protected from serious damage during the seedling and critical growth stages when most of the damage apparently occurs.

Total bromide content of the shelled peanuts appears to be below that which would be harmful, especially at the 0.5 and 1.0 gallon per acre rates. The peanut hay, however, probably should not be fed to milk cows or to animals being finished for slaughter. It is felt that further studies should be made before using hay from treated fields as feed.

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REACTION OF WATERMELON VARIETIES TO ROOT-KNOT NEMATODES

N. N. Winstead and R. D. Riggs[1]

Summary

Each of the 83 watermelon varieties and lines tested were susceptible to Meloidogyne javanica, M. incognita incognita, M. incognita acrita, and M. arenaria arenaria. All the varieties tested were resistant to M. hapla; however, a slight amount of reproduction occurred on all varieties.

Watermelon, Citrullus vulgaris, is often seriously damaged in North Carolina by root-knot nematodes. Bessey (1) reported that plants of one of the strains of watermelon x citron bred by Orton for wilt resistance were resistant to root-knot. Since that time most reports have indicated that watermelon is resistant to Meloidogyne hapla Chitwood, 1949, but susceptible to M. incognita incognita (Kofoid and White, 1919) Chitwood, 1949; M. incognita acrita Chitwood, 1949; M. arenaria (Neal, 1889) Chitwood, 1949, and M. javanica (Treub, 1885) Chitwood, 1949. Sasser (3) reported that Dixie Queen, Congo and Hawkesbury were resistant to M. hapla and that Dixie Queen was susceptible to the other four species and subspecies. Thomason (4) reported that Striped Klondike (Wilt Resistant) was resistant to M. hapla, susceptible to M. javanica, and only moderately susceptible to M. incognita acrita. Gaskin and Crittenden (2) found that Charleston Gray, Congo, Dixie Queen, Fairfax, Early Kansas, Florida Giant, Honey Cream, Hoosier Black, Northern Hybrid, Rhode Island Red and Wilt Resistant Ironsides were all resistant to M. hapla.

In this investigation 83 varieties and breeding lines of watermelon and citron were tested to determine their reaction to five Meloidogyne species and subspecies collected in North Carolina.

MATERIALS AND METHODS

Inoculum was increased from single eggmass cultures on Homestead tomato plants using techniques previously described (4). Infested soil containing tomato roots bearing eggmasses was used as inoculum. Five seeds were planted directly into 4-inch clay pots containing inoculum. Four pots of each variety were tested in each of two tests. From one to six sources of seed of each variety were tested. Stock populations of M. incognita incognita, M. incognita acrita, M. javanica, M. arenaria arenaria, and M. hapla were obtained from Dr. J. N. Sasser for use in these tests. In addition, a population of M. incognita acrita obtained from eastern North Carolina on Charleston Gray watermelon was also used.

Root-knot ratings were made 2 months after inoculation using a 0, 1, 2, 3, 4, scale (5). Varieties falling into the 0 to 2 classes are considered resistant (a dissecting microscope was used in making the 0 or 1 class determinations).

EXPERIMENTAL RESULTS

The varieties tested and their reactions to the root-knot nematode populations are presented in Table 1. Little or no differences were observed in the reactions of plants of the same varieties from different sources. All lines and varieties tested were susceptible to each of the populations of M. incognita incognita, M. incognita acrita, M. arenaria arenaria, and M. javanica. No differences in pathogenic reaction were noted between the two populations of M. incognita acrita; however, the population obtained from Sasser appeared to be somewhat more virulent than the population obtained from watermelon in eastern North Carolina. All the varieties tested were resistant to M. hapla; however, slight reproduction was noted on each of the varieties tested.

[1] Associate Professor, North Carolina State College and Assistant Professor, University of Arkansas, respectively.

Table 1. Reaction of watermelon varieties to root-knot nematodes (Meloidogyne spp.).

Variety[b]	Susceptibility ratings[a]					
	M. incognita incognita	M. incognita acrita[c]	M. incognita acrita	M. javanica	M. arenaria arenaria	M. hapla
Black Kleckly	4	4	4	4	4	1
Blacklee	4	3	4	3	4	1
Black Seeded Klondike	4	3	4	4	3	1
Blackstone	4	4	4	4	4	1
Blue Watson	4	4	4	3	3	1
Blue Wonder	4	3	4	4	3	1
California Honey	4	4	4	3	3	1
Cannon Ball Special	4	4	4	3	3	1
Charleston Gray	4	4	4	4	4	1
Chilean Black	4	4	4	3	4	1
Chilean Black Seeded	4	4	4	4	3	1
Citron	3	3	3	3	3	1
Cletex	4	4	4	3	4	1
Coles Early	4	4	4	3	4	1
Colorado Preserving Citron	4	4	3	4	4	1
Congo	4	3	4	3	4	1
Cut Red Watson	4	4	4	4	4	1
Darlington	3	4	4	3	4	1
Dixie Queen	3	3	4	3	3	1
Dude Creek	4	4	4	4	4	1
Early Canada	4	4	4	4	3	1
Early Kansas	4	4	4	4	4	1
Fairfax	4	3	4	4	3	1
Ferry's Peerless	4	4	4	3	4	1
Florida Favorite	4	4	4	4	4	1
Florida Giant	4	3	4	3	3	1
Fordhook Early	4	4	4	3	3	1
Garrison	4	3	4	3	4	2
Georgia Rattlesnake	4	4	4	4	3	1
Golden Honey	4	3	4	4	3	1
Graystone	4	4	4	4	4	2
Halberths Honey	4	3	4	3	4	1
Harris' Earliest	4	4	4	3	4	1
Hawksbury	4	4	4	4	3	1
Hope Diamond	4	4	4	4	4	1
Honey Cream	4	3	4	4	3	1
Ice Cream	4	3	4	4	3	1
Irish Gray	4	3	4	4	4	1
Ironsides	4	4	4	3	4	1
Kleckly No. 6 W. R.	4	3	4	4	4	1
Kleckly Sweet	4	4	4	4	4	1
Kleckly Sweet Improved	4	4	4	4	4	1
Klondike Black Seeded	4	3	4	3	3	1
Klondike R 7	4	3	4	4	3	1
Klondike Striped Blue Ribbon	4	4	4	4	4	1
Klondike Striped Wilt Resistant	4	4	4	3	3	2
Ledman	4	3	4	3	3	1
Leesburg	4	3	4	4	4	1

Table 1. Cont.

Variety[b]	Susceptibility ratings[a]					
	M. incognita incognita	M. incognita acrita[c]	M. incognita acrita	M. javanica	M. arenaria arenaria	M. hapla
Long Luscious Golden Honey	4	3	4	4	3	1
Miles	4	3	4	4	4	1
Monte Cristo	4	3	4	3	4	1
Mountain Hoosier	4	4	4	4	3	1
Nancy Hawks	4	4	4	3	4	1
Northern Sweet	3	4	4	4	4	1
N. C. 11[d]	4	4	4	4	4	1
N. C. 20[d]	3	4	4	4	4	1
N. C. 21[d]	4	3	4	4	4	1
N. C. 22[d]	4	3	4	4	4	1
N. C. W-695[d]	4	4	3	4	3	1
New Hampshire Midget	4	3	4	4	4	1
Norman Parker	4	4	4	4	4	1
Preserving Citron	4	3	4	3	4	1
Purdue Hawksbury	3	3	4	4	4	1
Red Heart Stone Mt.	4	4	4	4	4	1
Rhode Island Red	4	4	4	4	4	1
Snyder	4	4	4	3	3	1
Stone Mountain	4	4	4	4	4	1
Stone Mountain No. 5	4	3	4	3	3	1
Sugar Baby	4	3	4	3	3	2
Sunnybrook Hydrid	4	4	4	3	3	1
Super Black Diamond	4	3	4	4	3	1
Sweetheart	4	3	4	4	3	1
Takii Gem	4	4	4	4	3	1
Tendersweet	4	3	4	3	3	1
Texas Yellow Meat	4	4	3	4	3	1
Thurmond Gray	4	4	4	3	3	1
Tom Watson	4	4	4	3	3	1
W. R. Congo	4	4	4	3	4	1
Wilt Resistant Dixie Queen	4	3	4	4	4	1
Winona	4	3	4	4	4	1
Winter Queen	4	4	4	3	4	1
Winter King and Queen	4	4	4	3	3	1
Yellow Belly Black Diamond	4	4	4	4	4	1

[a]Susceptibility Rating: 0 -- no mature females; 1 -- very light infection, an occasional female eggmass; 2 -- light infection; 3 -- moderate galling, eggmasses moderately abundant; 4 -- severe infection with eggmasses very abundant. A dissecting microscope was used in making the 1 class determinations. Varieties falling into the 3 and 4 classes are considered susceptible.

[b]Seed were obtained from Associated Seed Growers, Inc., T. W. Woods & Sons, Inc., Simpson Nursery Company, Kilgore Seed Company, W. Atlee Burpee Co., Ferry Morse Seed Co., and Joseph Harris Company, Inc.

[c]Culture obtained from watermelon.

[d]North Carolina breeding lines 11 (susceptible to wilt and anthracnose, C. lagenarium races 1, 2, and 3); 20, 21, 22 (midget types resistant to wilt and anthracnose, C. lagenarium races 1 and 3); W-695 (citron resistant to C. lagenarium, races 1, 2, and 3).

DISCUSSION

Because Bessey (1) reported resistance to root-knot nematodes in Fusarium wilt resistant progeny from one of the crosses of citron x watermelon made by Orton, a large number of Fusarium wilt resistant as well as susceptible varieties were included in these tests. On the basis of these results it appears that Bessey was evaluating material for resistance to *M. hapla* or that he was working with pathogenically different populations from those included in this study. This latter explanation is plausible since Thomason (4) reported that the population of *M. incognita acrita* used in his studies did not incite severe root-knot on Striped Blue Ribbon (Wilt Resistant) plants, while both populations of *M. incognita acrita* used in this study caused severe root-knot on this variety.

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STUDIES OF THE NEMATODE, CRICONEMOIDES XENOPLAX, ON PEACH

B. F. Lownsbery

Summary

Criconemoides xenoplax is often the plant parasitic nematode species occurring in greatest numbers in peach orchards in Merced County, California. Nematicidal preplanting soil fumigation improves the growth of peach replants in these orchards. Greenhouse studies of the effect of C. xenoplax on peach seedlings have been inconclusive, but they do show that this nematode differs in its ecological requirements from the common peach parasite, Meloidogyne incognita acrita. If C. xenoplax injures peach in Merced County, the greatest injury probably occurs in the winter, or spring, when this nematode is at its highest population density.

A ring nematode, Criconemoides xenoplax Raski 1952, is a common plant parasitic species, and often the predominant one occurring around peach roots in the sandy soils of Merced County, California. It occurs around both S-37 and Lovell rootstocks, and is present in population densities as high as 12 nematodes per gram of soil. Peach roots in infested orchards often display the stubby fasciculation characteristic of injury by ectoparasitic nematodes. Growth responses to preplanting soil fumigation are commonly noted by growers in the area.

C. xenoplax has also been reported to occur around peaches in New Jersey (10). Chitwood (2) found large populations of C. simile Cobb 1918 parasitizing declining peaches in Maryland and North Carolina. It is apparently possible that these reports all deal with one and the same species of Criconemoides (9, see p. 89). Disease has been produced in Lovell peach seedlings by addition of soil screenings containing C. simile (3, 4). The number of nematodes in the screenings was not reported, and separate control additions of other organisms associated with the nematodes were not included.

EFFECTS OF NEMATICIDAL PREPLANTING SOIL FUMIGATION IN AN ORCHARD INFESTED WITH C. XENOPLAX

Shell "DD" (dichloropropene-dichloropropane mixture) and untreated control treatments were included by plant pathologist W. H. English in a 1957 field trial of effects of various treatments on incidence of peach canker in a replanted peach orchard at Atwater, California. C. xenoplax was the predominant plant parasitic nematode throughout this orchard. However, lower populations of Pratylenchus vulnus Allen and Jensen 1951, and Meloidogyne javanica javanica (Treub) Chitwood 1949, were also present. The "DD" treatment was applied in January at the rate of 40 gallons per acre. This dosage gave a good nematode kill in the sandy (moisture equivalent 4 percent; pH 5) soil (Table 1). Red Haven peaches on S-37 rootstocks were planted at treated and untreated sites the following spring. At the end of the first growing season, 92 trees unaffected by canker were available for measurement, half of them grown at treated sites, the other half at untreated sites. Trunk circumferences of trees grown at treated sites were significantly greater than those of trees grown at untreated sites (Table 1). Because of the occurrence of P. vulnus and M. javanica javanica in this orchard, and because "DD" treatment may also produce other changes than those in nematode population density (e.g., 8), further evidence was required to judge the effect of C. xenoplax on peach. This was sought in the greenhouse experiment reported below.

THE EFFECT OF C. XENOPLAX AND M. INCOGNITA ACRITA CHITWOOD 1949, ON PEACH SEEDLINGS

Lovell peach seedlings, obtained by embryo culture (6) and selected for uniformity, were supplied by pomologist C. J. Hansen. A fine sandy loam (moisture equivalent 12 percent; pH 7.5) was used as the growing medium. Inoculum of C. xenoplax was obtained by screening soil from an infested peach orchard (5). Second-stage larvae of M. incognita acrita were obtained from egg masses (7).

Table 1. Peach tree trunk circumferences, and population densities of Criconemoides xenoplax at "DD" treated sites and untreated sites; Atwater, California.

	Trunk circumference (mm.)	C. xenoplax per gram of soil [a]
Sites treated with "DD" at 40 gals. per acre	128	0.01 ± 0.01 [b]
Untreated sites	118	2 ± 1
L.S.D. at the 5% level	8	
L.S.D. at the 1% level	10	

[a]C. xenoplax was recovered by a modification of Cobb's screening-gravity method (5) from composite samples taken to a 3-foot depth in the root zones of treated and untreated trees with a Viehmeyer (12) soil tube.
[b]The mean with its standard error.

Table 2. Lovell peach seedling weights and final nematode population densities after 3 months' growth of the seedlings with six population densities of Criconemoides xenoplax and Meloidogyne incognita acrita.

Initial nematode density (Nematodes /gm. soil)	Final seedling weight (gms.)	Final nematode density (Nematodes/gm of soil and roots)
C. xenoplax		
0 ± 0	41	0 ± 0 [a]
0.005 ± 0	61	0 ± 0
0.05 ± 0.008	48	0.001 ± 0.001
0.5 ± 0.08	60	0.05 ± 0.01
5.0 ± 0.78	55	0.4 ± 0.04
50.0 ± 7.76	58	3.6 ± 0.1
L.S.D. at the 5% level	21	
L.S.D. at the 1% level	29	
M. incognita acrita		
0 ± 0	44	0 ± 0
0.005 ± 0	48	0.02 ± 0.01
0.05 ± 0.005	34	3.2 ± 0.6
0.5 ± 0.05	41	13.2 ± 5.7
5.0 ± 0.52	26	8.9 ± 2.9
50.0 ± 5.23	12	5.2 ± 2.1
L.S.D. at the 5% level	14	
L.S.D. at the 1% level	19	

[a]The mean with its standard error.

Table 3. Population density of Criconemoides xenoplax in soil samples taken from an Atwater, California orchard at nine different times of the year.

Date of sampling	C. xenoplax per gram of soil
November 15, 1956	0.3
December 17, 1956	0.4
January 16, 1957	1.4
February 15, 1957	1.2
March 15, 1957	2.4
April 18, 1957	1.1
July 19, 1957	0.4
August 16, 1957	0.6
September 20, 1957	0.7
L.S.D. at the 5% level	1.0
L.S.D. at the 1% level	1.3

Nematodes were added to the 6-inch pots of soil immediately before planting the peach seedlings. Two hundred milliters of tap water containing sufficient volume of nematode suspension to give the desired number of nematodes were stirred into the soil. Zero levels of population density were obtained by passing the nematode suspension repeatedly through a screen with 43-micron openings until the nematodes were removed. Thus the zero levels received any bacteria and fungi present in the nematode suspension.

Pots were arranged in four randomized blocks on a greenhouse bench, where temperature ranged from 20° to 31° and averaged 26° C during the course of the experiment.

After 3 months' growth, fresh weights of peach seedlings were obtained and final nematode populations were determined. C. xenoplax was recovered by screening. M. incognita acrita was recovered from soil by the Baermann method (1), and from peach roots by examination of blendorized aliquots.

Final weights of peach seedlings grown with M. incognita acrita were inversely proportional to the number of nematodes added initially (Table 2, Fig. 1).

Growth of peach increased the population density of M. incognita acrita, except where the initial population was so high that the seedlings were severely stunted (Table 2).

Final weights of peach seedlings grown with C. xenoplax were not related to the numbers of nematodes added initially (Table 2). The peach roots produced at the highest population density of C. xenoplax did have a shelf-like form different from all other root systems in the experiment (Fig. 2).

Populations of C. xenoplax declined from initial levels in all cases (Table 2), indicating that this nematode has different ecological requirements than were provided in this experiment, and different requirements from those of M. incognita acrita.

SEASONAL FLUCTUATION IN ORCHARD POPULATION DENSITY OF C. XENOPLAX

Soil around each of four peach trees on S-37 rootstocks at Atwater was sampled at each of the dates indicated in Table 3. The samples representing each tree were composites of four sub-samples taken to a 3-foot depth with a Viehmeyer soil tube (12) around the drip line of the tree. C. xenoplax was recovered by screening (5).

C. xenoplax populations were highest in winter and early spring, lowest in late summer

FIGURE 1. Representative Lovell peach roots produced at population densities of 0, 0.005, 0.05, 0.5, 5.0, and 50.0 Meloidogyne incognita acrita per gram of soil (left to right).

FIGURE 2. Lovell peach roots: grown with a population density of 50 Criconemoides xenoplax per gram of soil (right); grown under the same conditions but without C. xenoplax (left).

and fall (Table 3). These data are supported by results of less systematic sampling in other years.

If C. xenoplax injures peach trees in Merced County, the greatest injury probably occurs in winter and spring when the nematode is present in highest numbers. During the summer and fall C. xenoplax in Merced County orchards is exposed to higher temperatures and to periodic drying. The nematode is probably adversely influenced by one or both of these factors.

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AN ELECTRIC AUGER FOR NEMATOLOGICAL SOIL SAMPLING IN ORCHARDS

B. F. Lowrisbery, John T. Mitchell and Ernest E. Head

For a number of reasons, one of which is inherent tree variability, it is necessary to include a large number of trees in tests measuring effects of nematode control on tree growth. Soil in the root zone of each tree must be sampled to a depth of several feet to obtain a true measure of the number of nematodes affecting the tree. The problem of assaying nematode populations in orchard plots is, therefore, a formidable one. We have used the Viehmeyer soil tube[1] for this purpose, but we find that this tool requires greater time and effort than we have available. The electric auger described here enables two men to take a large number of 3-foot soil borings, at the rate of one boring per minute, without undue effort.

DESCRIPTION

A ship auger 3 feet long and 3/4 inch in diameter is powered by an electric drill motor equipped with a reducing gear assembly[2]. Auger and motor weigh 16 pounds. A masonry type drill point, 1 inch in width, welded to the auger tip (Fig. 1) provides clearance enabling the auger to be withdrawn from the soil easily. Electric power is furnished by a small (1500 Watts) electric generator[2] which is carried in the back of a pickup truck (Fig. 2). An auger of this

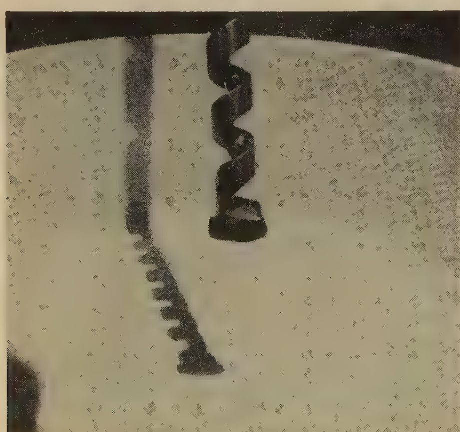

FIGURE 1. Close-up of auger tip.

FIGURE 2. Sampling a peach orchard with the electric auger.

type may also be powered by an automobile battery[3] if it is not used continuously for long periods.

USE

A plastic pan is placed on the ground at the spot where a sample is to be taken. The auger is driven through the center of the pan into the soil (Fig. 3). In some soils the sample drops off the auger into the pan as the auger is withdrawn. In others, it is necessary for a second person to dislodge the soil by running his fingers up the auger as it is withdrawn. Because the volume of soil obtained in a single boring is small (approximately 1/2 cup), it is possible to obtain composite samples of reasonable volume. After each sub-sample is taken, the soil is shifted to the side of the pan, and the pan is moved to the site of the next subsample. When all the sub-samples are in the pan, the composite sample is emptied into a labeled bag.

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[1] Viehmeyer, F. J. 1929. An improved soil sampling tube. Soil Science 27: 147-152.

[2] The drill motor and generator which we use were purchased from the Gen-a-matic Corporation, Van Nuys, California.

[3] Anonymous. 1959. Make-it-yourself soil tester. Better Farming Methods 31(2): 53.

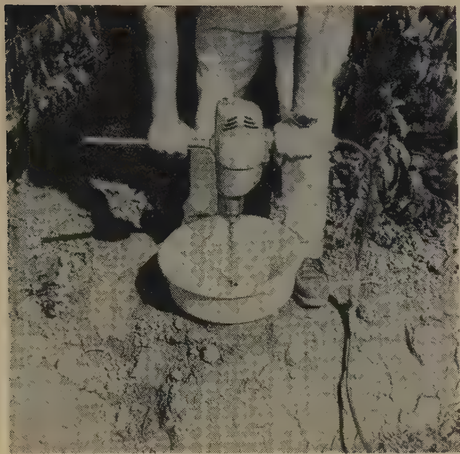

FIGURE 3. Close-up of auger and soil collection pan.

TEST OF DEPTH REPRESENTATION IN SOIL BORINGS OBTAINED WITH THE AUGER

Clay pipes 3 feet in length were filled with soil as follows:

A. The bottom halves of 10 pipes were packed with a well-mixed soil, infested with *Heterodera schachtii* Schmidt; then the top halves were packed with nematode-free soil.

B. The bottom halves of 10 pipes were packed with nematode-free soil; then the top halves were packed with the soil infested with *H. schachtii*.

C. Ten pipes were packed entirely with the soil infested with *H. schachtii*.

Tubes packed in these three ways were placed on end and sampled with the electric auger as we have described. Cysts of *H. schachtii* were recovered from the soil samples by screening, and counted. The mean number of cysts recovered from series A did not differ significantly from the mean number recovered from B, nor from the mean of one-half of the number recovered from C. This indicates that the soil samples obtained with this auger truly represent the 0- to 3-foot depth.

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NORTHWESTERN APPLE TREE ANTHRACNOSE REPORTED FROM MICHIGAN[1]

Evan H. Pepper[2]

Early this year (1959), Golden Delicious apples from Laingsburg, Michigan, held in refrigerated storage, developed characteristic "bull's-eye" spots (Fig. 1). At about the same time similarly rotted apples of the same variety were received from a Maine orchard. Isolations from both lots of apples yielded the same fungus, identified as Gloeosporium sp. on the basis of macroscopic appearance, conidial size and shape, and temperature-growth relations. Golden Delicious, Stayman, Steele's Red (Red Canada), Winesap, Wealthy, Dark Red Jonathan, Wagener, MacIntosh, and Cortland apples inoculated with spore suspensions of both Maine and Michigan isolates developed typical "bull's-eye" rots. The orchard from which the diseased fruit was obtained disclosed numerous cankers throughout 20 acres of Golden Delicious trees (Fig. 2). MacIntosh and Jonathan trees were canker-free. The infected trees were 30 years old and vigorous. The grower first observed the fruit-rot about 6 years ago but did not suspect its fungal nature because of the longer period between harvest and the first signs of decay in storage.

FIGURE 1. Bull's-eye rot on naturally infected Golden Delicious apples.

Cankers collected from the orchard and placed in a moist chamber have produced acervuli and conidia which are typical of Gloeosporium malicorticis Cordley (2). The perfect stage (Neofabraea malicorticis (Cord.) Jackson) has not yet been found and a search will be made in the fall.

[1] Journal Article No. 59-14, Department of Botany and Plant Pathology. The writer gratefully acknowledges the receipt of specimens and continued interest of W. Clerx and Dr. D. Dewey, Department of Horticulture, Michigan State University.
[2] Graduate Research Assistant, Department of Botany and Plant Pathology, Michigan State University.

FIGURE 2. Northwestern apple tree anthracnose cankers on Golden Delicious twigs.

The distribution of the disease in Michigan has not yet been determined and surveys of the larger apple-growing areas are now underway. This disease has not previously been recorded in Michigan. This, together with Anderson's report (1) apparently constitutes the only recorded occurrence of apple tree anthracnose in the Midwest.

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THE POSSIBLE RELATIONSHIP BETWEEN ASPERGILLUS FLAVUS AND ALBINISM IN CITRUS[1]

Richard D. Durbin[2]

Virescent or albino seedlings often occur in citrus seedbeds. These plants lack chlorophyll to some degree, varying from only the veins or portions of the mesophyll affected to the entire plant. Albinism can be controlled either by treatment of the seed with fungicides or inorganic salts of heavy metals (3, 4) or by removal of the seedcoats (5). It has been suggested that a microorganism may be responsible for the disease (1, 4). Koehler and Woodworth have demonstrated that a similar abnormality of corn can be caused by either *Aspergillus flavus* Link or *A. tamarii* Kita (2). Since these fungi are widespread, particularly in tropical and subtropical soils and as air-borne contaminants (6), the possible relationship between the two diseases was investigated.

Dent corn inbred line W22 was inoculated by soaking seeds in a spore suspension of a number of clones of *A. flavus*[3] and germinated under conditions favorable for the development of chlorophyll-deficient seedlings. The fungus was isolated from albino plants and reinoculated into corn. Clones of the fungus isolated as a result of this second selection produced 25 to 75 percent virescent plants when inoculated singly into corn.

Subsequently, surface-sterilized seeds[4] of *Citrus sinensis* (L.) Osbeck (sweet orange) and *C. paradisi* Macf. (grapefruit) were inoculated with these clones and germinated in autoclaved soil. The results are presented in Table 1. Both sweet orange and grapefruit seed which had been inoculated with *A. flavus* produced albino seedlings, whereas seed not inoculated produced no albino seedlings (Fig. 1). The fungus could be isolated from the seedcoats, but not from the remainder of the seedling. This is in accordance with the observation that removal of the seedcoats prior to planting can eliminate the disease (5).

Table 1. Effect of *Aspergillus flavus* on the incidence of albinism in two species of Citrus.

Species	Percent germination[a]		Percent albinism	
	Inoc.	Non-inoc.	Inoc.	Non-inoc.
C. sinensis	60	50	43	0
C. paradisi	94	98	28	0

[a] Fifty seeds per treatment. Data taken 3 months after planting.

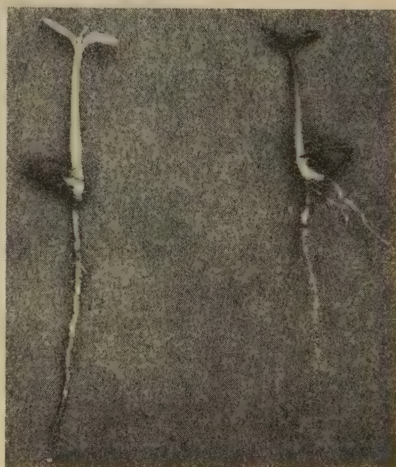

FIGURE 1. Effect of seed inoculation with *A. flavus* on seedlings of *C. paradisi*. Left, inoculated; right, non-inoculated.

These results indicate that the fungus grows primarily as a saprophyte on the seedcoats and produces a metabolite which, when taken up by the germinating seed, inhibits chlorophyll biosynthesis. While this experiment does not prove that albinism in citrus seedlings is caused by *A. flavus* under natural conditions, the results strongly support the presumption that it is so caused.

[1] Paper No. 4194, Scientific Journal Series, Minnesota Agricultural Experiment Station.
[2] Assistant Professor, Department of Plant Pathology and Botany, University of Minnesota.
[3] Courtesy of C. M. Christensen, Department of Plant Pathology and Botany, University of Minnesota.
[4] Courtesy of E. F. Frolich and G. F. Ryan, Department of Horticultural Science, University of California, Los Angeles.

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LATE BLIGHT RESISTANCE OF SELECTED POTATO SEEDLINGS HIGHLY RESISTANT TO RING ROT

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Abstract

Five ring-rot- and late-blight-resistant potato seedlings and seven commercial varieties were planted in a field where environmental conditions were favorable for development of late blight. The five ring-rot-resistant seedlings had no, or only slight, late-blight foliage infection and no tuber decay developed throughout the entire season. In contrast, all the commercial varieties were killed or badly defoliated by the disease. All the commercial varieties except Sebago were susceptible to tuber decay, which varied from 3 percent for the Cherokee variety to 57 percent for the Chippewa variety. Late blight reduced the yield significantly in the commercial varieties by defoliating the plants and by rotting the tubers in the soil. Sebago exhibited a high degree of field resistance. Although immune from race 0, the Kennebec and Cherokee varieties were badly defoliated by late blight and the yields were reduced significantly. These varieties also were susceptible to tuber decay in the soil.

INTRODUCTION

The control of late blight, caused by Phytophthora infestans (Mont.) d By., is an important problem always confronting potato growers in Maine. This disease still causes large losses in spite of extensive spraying. Late blight reduces the crop principally by destroying the foliage of the potato plants and by causing large losses from late-blight-tuber rot (1, 2, 3, 4). A survey in Aroostook County in 1957 showed that 10 to 15 percent of the potatoes in bins had late-blight-tuber rot, and in some bins 25 to 35 percent of the tubers were infected (2 and unpublished data).

There is a need for commercial varieties resistant to late-blight infection on both the foliage and tubers. It would be desirable if the late-blight-resistant varieties were also highly resistant to or immune from ring rot, caused by Corynebacterium sepedonicum (Spieck. & Kotth.) Skapt. & Burkh., and other diseases.

This paper presents the results of tests conducted in 1957 on a number of ring-rot-resistant selections to determine their resistance to late blight under optimum field conditions for infection. The seedlings selected failed to show ring-rot infection after inoculation for three or more years and were known to be resistant to one or more races of late blight.

METHOD OF PROCEDURE

Five ring-rot-resistant seedlings possessing factors for resistance to late blight and seven commercial varieties were planted in a field of Washburn loam soil known to provide conditions favorable for the development of late-blight-tuber rot. The Kennebec and Cherokee varieties were included because their foliage is immune to race 0 of the late-blight fungus, and their tubers are considered resistant to tuber rot of the same race, but not to the tuber rot caused by race 1 (7). Sebago was included because it possesses field resistance to the late blight races occurring in Maine, and its tubers are resistant to late-blight-tuber rot (5).

Fungicidal sprays for late blight control were omitted. The degree of blight infection was estimated at different times during the growing season. Harvest was delayed until after a killing frost in order to avoid transmission of foliage infection to the tubers. At harvest time the tubers were examined for late-blight rot that had occurred during the growing season and the yields of marketable tubers were obtained.

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RESULTS

Foliage Infection

The 1957 growing season was one of the most favorable for the development of late blight in the history of Maine. The disease was observed in many potato cull piles and on June 3 and June 22 was found in several commercial fields near Presque Isle. Weather conditions were ideal for rapid dissemination of the late-blight fungus throughout the growing season.

FIGURE 1. Late-blight-resistant seedling B 3201-38 (left) and susceptible Green Mountain (right) grown under favorable conditions for late blight. Seedling B 3201-38 had only a trace of late blight infection throughout the entire growing season. It also had no ring rot infection in other experiments.

Table 1. Late-blight foliage infection of varieties and seedlings for different dates, Maine, 1957.

Seedling or variety	Parentage	Percent late blight foliage infection on: Aug. 2	Aug. 8	Aug. 20	Sept. 2	Sept. 12
B 3201-38	B 922-3 x B 919-15	0	0	Trace	Trace	Trace
B 4157-1	B 355-24 x B 919-15	0	0	Trace	Trace	Trace
B 3879-3	Ac. 25955 x B 355-24	0	0	0	0	0
Kennebec	B 127 x 96-56	0	0	2	24	77
B 3686-1	B 931-2 x Ac. 25953	0	0	0	Fleck[a]	Fleck[a]
B 3102-3	Mohawk x Merrimack	0	0	12	41	77
Cherokee	96-56 x 528-170	0	0	6	75	Mature
Sebago[b]	Chippewa x Katahdin	0	6	16	41	75
Katahdin	40568 x 24642	Trace	10	40	100	100
Green Mountain	Dunmore x Excelsior	Trace	10	77	100	100
Chippewa	40568 x 24642	Trace	12	88	100	Mature
Russet Rural	--- ---	Trace	12	88	100	100

[a]Apparently infected, but no positive sporulation

[b]Field resistant to all genotypes.

The extent of late-blight-foliage infection occurring on the different seedlings and varieties on five dates is given in Table 1. Disease measurements were made according to the method of Horsfall and Barratt (6). The susceptible varieties Katahdin, Green Mountain, Chippewa and Russet Rural each had a trace of infection on August 2. Inoculations of genotype-test plants in the greenhouse with late blight cultures from the field showed that race 4 predominated and appeared first. The disease progressed rapidly in the field and completely destroyed the foliage of the susceptible varieties by the first week in September. Sebago, although infected

early in the season, possessed considerable field resistance, which delayed the progress of the disease as compared with the other susceptible commercial varieties. This varietal reaction has been noted previously in Maine (5).

The vines of Cherokee and Kennebec, which are immune from race 0, were found to be infected on August 20 and were badly defoliated by September 2 and 12. Kennebec, however, had less infection than Cherokee. Seedling B 3102-3, possessing a low degree of field resistance like Cherokee and Kennebec, was severely defoliated toward the end of the season.

Seedling B 3879-3 was found to be highly resistant and showed no foliage infection throughout the test. Seedling B 3686-1 also was highly resistant and only small spots with no detectable fungus sporulation developed toward the end of the season. Seedlings B 3201-38 and B 4157-1 likewise were very resistant. They had occasional blighted spots on August 20, but the disease failed to spread. These two seedlings, as well as B 3686-1, appear to have high field resistance to the late-blight fungus and in addition possess immunity from several specific races of the organism. Figure 1 shows a comparison of a ring-rot- and late-blight-resistant seedling B 3201-38 with susceptible Green Mountain grown under conditions favorable for late-blight infection.

Tuber Infection

The percentages of tuber decay of the seedlings and varieties in the test are presented in Table 2. The five seedlings with both ring-rot and late-blight resistance had no tuber decay when the test was harvested, indicating that they were probably resistant. At harvest time 13 percent of the Kennebec tubers showed tuber rot, indicating that the tubers of a resistant variety may become susceptible to some races of the organism. The vines of the Cherokee variety

Table 2. Late-blight tuber infection and yields of marketable tubers per acre of seedlings and varieties[a], Maine, 1957.

Variety or seedling	Tuber infection[b] (percent)	Yield per acre[a] (2-inch minimum) Bushels	Cwt.
B 3201-38	0	518	311
B 4157-1	0	474	284
B 3879-3	0	410	250
Kennebec	13	282	169
B 3686-1	0	259	155
B 3102-3	0	174	105
Cherokee	3	149	89
Sebago	0	136	81
Katahdin	18	67	40
Green Mountain	50	33	20
Chippewa	57	5	3
Russet Rural	18	0	0
L.S.D 5%		59	35
L.S.D 1%		80	48

[a]Seedlings resistant to both ring rot and late-blight infection.

[b]Tuber infection at time crop was dug.

died early in the season, thus lessening the chance of infection, with the result that only 3 percent of the tubers showed tuber rot.

All the susceptible varieties had abundant tuber decay which varied from 18 percent for Katahdin and Russet Rural varieties to 50 and 57 percent for Green Mountain and Chippewa, respectively. This tuber decay resulted from soil inoculations by late blight conidia which were washed off infected foliage by the action of rainwater.

Although the foliage of Sebago is generally known to have field resistance to late blight, its vines, like those of most field resistant varieties, became severely infected toward the end of the growing season. Its tubers, however, showed no indications of tuber rot.

Effect on Yield

Late blight significantly reduced the yields for the commercial varieties Katahdin, Green Mountain, Chippewa, and Russet Rural (Table 2). Russet Rural produced no marketable tubers.

Yield reductions of the susceptible varieties resulted from extensive defoliation and tuber decay caused by the late-blight fungus. Sebago, although susceptible to foliage infection by all the races of late blight, possessed considerable field resistance and produced significantly greater yields than the other susceptible varieties. The Kennebec and Cherokee varieties, which are immune from late blight race 0, were severely attacked and their yields were reduced.

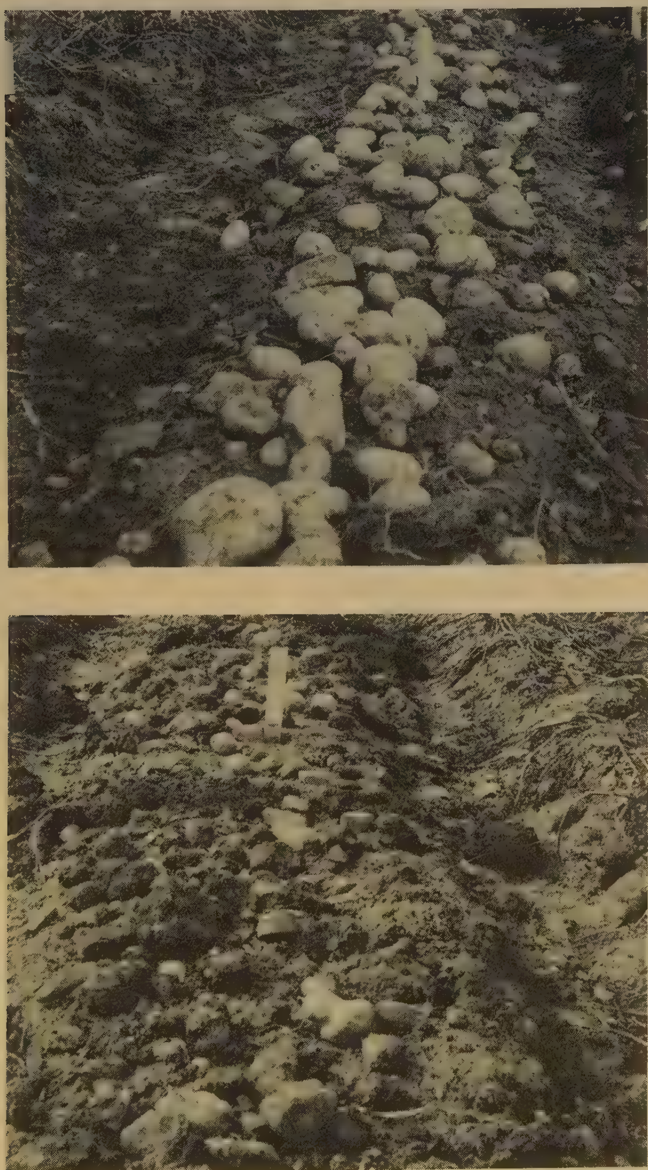

FIGURE 2. Healthy tubers of seedling B 3201-38 (upper) and Green Mountain (lower) in an unsprayed plot. B 3201-38 yielded 311 cwt. and Green Mountain 20 cwt. of marketable tubers per acre.

The three higher yields were produced by the late-blight-resistant seedlings B 3201-38, B 4157-1 and B 3879-3. Although the foliage and tubers of seedling B 3686-1 are resistant to late blight, it produced a small yield in comparison with yields of the other resistant seedlings. Figure 2 shows the yields produced by seedling B 3201-38 and Green Mountain when grown under conditions favorable for the rapid spread of late blight.

DISCUSSION AND CONCLUSIONS

The results of this experiment show that some desirable ring-rot-resistant seedlings are also highly resistant to or immune from one or more races of the late blight fungus. The five seedlings included in this test failed to develop ring rot in previous experiments when their cut seed pieces were inoculated by dipping in a heavy slurry of the bacteria. Although not sprayed with a fungicide, four of the five seedlings had very little or no infection from late blight on their foliage or tubers when grown under soil and weather conditions favorable for tuber decay and ideal for the development of late blight. The susceptible commercial varieties, except Sebago, were badly infected and produced small crops when grown under the same conditions.

Although immune from late blight race 0, the vines of Kennebec and Cherokee were severely defoliated and their tubers were susceptible to tuber decay. This type of resistance apparently is of little value during seasons favorable for the rapid spread of late blight if the crop is not sprayed with a fungicide.

Field resistance to the different races of late blight found in Maine, as exhibited by Sebago, should be valuable in the development of new varieties. The experiment showed that through the work of the National Potato-Breeding Program it has been possible to develop varieties combining high resistance to ring rot with resistance to several races of the late blight fungus.

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MAINE AGRICULTURAL EXPERIMENT STATION AND UNITED STATES DEPARTMENT OF AGRICULTURE

MICROBIAL ASSOCIATIONS IN THE FUSARIUM ROOT ROT OF BEANS[1]

Otis C. Maloy[2]

Summary

Studies were made of the organisms observed microscopically in diseased bean roots and those isolated from diseased roots. The effects of different crops or amendments on the incidence of these organisms was investigated. The organisms observed microscopically were generally different from those isolated. Both qualitative and quantitative differences were found in the types of fungi in diseased roots in relation to age of plants, type of soil amendment, and distribution of fungi in root rot lesions.

Microscopic examinations of diseased roots often reveal microorganisms not easily obtained by isolation techniques (5). The majority of the organisms isolated from diseased tissue are not involved in the initiation of disease but colonize the substrate that is made available by the death of the plant or plant parts. The experiments discussed in this paper involved studies of 1) the organisms observed microscopically in or on bean roots, and 2) fungi isolated from bean roots.

MATERIALS AND METHODS

Roots were prepared for examination by boiling in 10 percent KOH for several minutes and staining in 0.5 percent acid fuchsin in lactophenol. Fungi were isolated by plating sections of diseased roots on PDA acidified by the addition of 1 drop of 50 percent lactic acid per 20 ml of medium.

RESULTS

Microscopic Studies

An examination of 2750 roots revealed the presence of a number of organisms, most of which were similar to the root inhabiting organisms observed in other plants (5). The organisms consistently observed included: Thielaviopsis basicola (Berk. & Br.) Ferr., Rhizoctonia solani Kuehn, Olpidium spp., nematodes, the "Rhizoctonia mycorrhizal fungus", and the "Phycomycetous mycorrhizal fungus" which has been identified as Pythium ultimum Trow (4). In addition, fruiting structures of many other organisms were observed.

Roots selected at various times after planting from beans growing in a "root rot plot" heavily infested with the root rot Fusarium (3) were examined. The occurrence of most of the organisms observed increased to a peak at some time during the season and then declined (Table 1). In a field study of bean roots taken 10 weeks after planting, the various organisms generally were recorded most frequently from beans grown following beans or corn and least frequently after cropping to alfalfa or clover. No consistent crop effect was observed in the greenhouse study (Table 2) although the highest incidence of a particular microorganism was generally observed following Red Kidney bean cropping or manure amendment.

Isolation Studies

In isolations made from bean roots at various times after planting (Table 3), the primary root rot fungus, Fusarium solani f. phaseoli (Burk.) Snyd. & Hans., was isolated with about the same regularity at all of the sampling dates. The fungus most commonly isolated was

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Table 1. The frequency of occurrence of various microorganisms in bean roots at various times after planting as determined by microscopic examination.

Organism	Percentage of roots invaded by the indicated organism at different times after planting (weeks)							
	1	2	3	4	6	8	10	12
Thielaviopsis basicola	0	2	6	24	18	34	22	8
Rhizoctonia solani	8	4	16	30	4	20	2	20
Olpidium spp.	2	52	42	26	28	18	12	18
Phycomycetous mycorrhizal fungus	2	6	8	34	26	26	40	24
Rhizoctonia mycorrhizal fungus	0	0	2	2	2	0	0	0
Thielavia spp.	0	0	0	8	10	10	10	2
Nematodes	10	46	72	12	34	16	0	34
Unidentifiable mycelium	54	60	72	66	78	72	80	84
Alternaria spp.[a]	-	+	+	-	+	+	+	+

[a] Recorded as present or absent from a sample.

Table 2. The frequency of occurrence of various microorganisms in roots of 6-week-old Red Kidney bean plants following various crops or organic amendments in a "greenhouse" rotation experiment as determined by microscopic examination.

Organism	Percentage of roots invaded by the various organisms in soil previously cropped or amended with the indicated crop or material						
	Red Kidney Bean	N-203[a] Bean	Alfalfa	Red Clover	Wheat	Manure	Sawdust
Thielaviopsis basicola	10.4	12.8	0.0	2.4	8.8	44.8	32.8
Rhizoctonia solani	8.0	4.0	4.0	0.0	0.8	0.0	0.8
Olpidium spp.	36.0	12.0	16.0	14.4	8.8	59.2	28.0
Phycomycetous mycorrhizal fungus	15.2	10.4	33.6	19.2	32.0	12.0	26.4
Rhizoctonia mycorrhizal fungus	16.0	16.8	5.6	1.6	2.4	0.8	4.8
Unidentifiable mycelium	64.8	56.0	76.8	68.8	70.4	31.2	60.8
Nematodes	34.4	22.4	38.4	60.0	37.6	24.0	19.2
Thielavia spp.	0.8	0.0	0.0	0.0	0.0	10.4	0.8
Corynespora spp.	20.0	6.4	1.6	6.4	1.6	1.6	8.0
Chlamydospores	4.0	4.0	9.6	6.4	5.6	3.2	0.8

[a] A bean with small, black seeds that is being used as a source of root rot resistance in breeding experiments at Cornell University.

Table 3. The frequency of occurrence of various fungi isolated from bean roots at different times after planting.

Fungus	Percentage of isolates obtained at indicated times after planting (weeks)					
	2	4	6	8	10	12
Fusarium solani f. sp. phaseoli	3.8	9.4	3.9	7.8	7.0	7.3
Fusarium oxysporum	45.3	33.3	31.4	33.3	38.6	29.1
Trichoderma spp.	7.6	11.1	11.8	2.0	5.3	0.0
Penicillium spp.	30.2	24.1	37.3	23.5	17.5	14.6
Aspergillus spp.	0.0	1.8	0.0	17.6	7.0	3.6
Mucor spp.	1.8	7.4	0.0	5.9	8.8	3.6
Alternaria spp.	1.8	0.0	3.9	2.0	0.0	1.8
Chaetomium spp.	3.8	0.0	0.0	2.0	0.0	1.8
Gliocladium spp.	0.0	0.0	3.9	3.9	10.5	32.7
Miscellaneous[a]	5.7	12.9	7.8	2.0	5.3	5.5
Total number of isolates	53	54	51	51	57	55

[a] Miscellaneous included members of the following genera: Pythium, Rhizopus, Cunninghamella, Rhizoctonia, Sclerotium, Monilia, Cephalosporium, Thielaviopsis, Coniothyrium, and Helminthosporium.

Table 4. The frequency of occurrence of various fungi isolated from moderately rotted bean roots (Root Rot Index = 66) at various distances from the margin of lesions.

Fungus	Percentage of isolates obtained at indicated distances from lesion margin that were identified as shown			
	0 cm	1 cm	2 cm	3 cm
Fusarium solani f. sp. phaseoli	3.0	14.0	11.6	7.1
Fusarium oxysporum	29.7	30.3	26.1	27.8
Alternaria spp.	20.3	4.7	3.1	1.3
Gliocladium spp.	4.2	16.8	15.2	16.9
Penicillium spp.	20.8	12.5	15.4	18.8
Aspergillus spp.	6.7	7.4	8.3	12.1
Mucor spp.	5.0	2.0	6.4	3.7
Trichoderma spp.	3.4	2.4	5.1	9.8
Rhizoctonia spp.	0.0	0.8	1.6	0.0
Thielaviopsis basicola	1.0	0.0	0.0	0.8
Miscellaneous	5.6	9.2	7.3	1.7
Total number of isolates	98	124	131	139

Fusarium oxysporum (sensu Snyder & Hansen). Penicillium spp. and Trichoderma spp. were isolated most frequently at the early sampling periods, Aspergillus spp. were more prevalent at intermediate times, and Gliocladium spp. were not isolated to any extent until 10 weeks after planting.

A representative sample of 214 fungus isolates was selected from 1059 isolates obtained from bean roots of various ages and was tested for pathogenicity on beans. Only the isolates that were identified as F. solani f. phaseoli caused any appreciable root rot and these could be placed into three groups based on relative pathogenicity. A study was made of the effect of mixtures of four bean root pathogens, F. solani f. phaseoli, R. solani, T. basicola, and Pythium sp., on root rot severity. None of the possible combinations resulted in more severe root rot than the bean Fusarium alone.

An attempt was made to group the fungus isolates on the basis of their ability to utilize carbon sources of varying degrees of complexity, following the scheme suggested by Burges (2). Isolates were compared in their ability to grow on agar media or in liquid media containing glucose, xylan, cellulose, or "native" lignin[3]. In general, the isolates occurring most abundantly at the earlier times (Penicillium and Trichoderma) produced their maximum growth only on glucose and xylan. Isolates obtained most frequently at the intermediate sampling times (Aspergillus and Alternaria) gave comparable growth on glucose, xylan, and cellulose. The isolates obtained in greatest numbers at the later sampling times yielded comparable growth on all four substrates. These results should be considered with certain reservations since great variations occurred among isolates of the same taxonomic group. Other qualifying factors are: 1) the isolation was somewhat selective in favoring those fungi having a rapid rate of growth on media with a relatively high sugar content, 2) the more readily assimilated substrates (glucose and xylan) may be exhausted more rapidly than others, and 3) the fungi may utilize ethanol from an ethanol-lignin complex rather than the lignin (6).

To determine the distribution of fungi in root rot lesions, isolations were made from 2-mm sections removed from 11-week-old bean plants. One section was taken from the upper margin of the lesion and others from points 1, 2, and 3 cm below that margin. The primary pathogen, F. solani f. phaseoli, was isolated infrequently from the margin, but this fungus comprised one out of seven colonies developing from sections taken 1 cm from the margin (Table 4). A high incidence of Alternaria spp. was recorded at the margin of lesions, with a sharp decrease in frequency as the distance from the margin increased.

DISCUSSION

The organisms observed in bean roots by microscopic examination include several that are known root rot pathogens, i.e., T. basicola, R. solani, and a fungus reported to be Pythium ultimum (4). Only the first of these appears to be consistently associated with necrotic tissue on bean roots. Except for two organisms, at some time in the growing season the occurrence of the organisms in bean roots reached a maximum that was followed by a decline in percentage of roots invaded. Several factors, soil temperature, soil moisture, age of plants, etc., may be responsible for this decline. A general agreement between greenhouse and field results was observed in the effects of cropping or amending soil on the frequency of occurrence of the different organisms.

The organisms isolated from bean roots were, in general, different types from those observed by microscopic examination. Of the organisms commonly observed microscopically only three, T. basicola, R. solani, and Alternaria spp., were isolated. These results emphasize the fact that the absence of a particular organism from the isolation plate should not be taken as final evidence that the organism is not present.

F. oxysporum was the fungus most frequently isolated from diseased bean roots but pathogenicity tests proved that only species morphologically similar to F. solani f. phaseoli caused any appreciable root rot on beans. In routine isolations, tissue is usually taken from the margin of lesions, but the root rot Fusarium was isolated 5 times as frequently from tissue 1 cm from the margin of lesions as from the marginal tissue. There is a possibility that the root rot fungus excretes a substance that is toxic to plant cells and causes a necrosis in advance of the penetrating hyphae.

[3] "Native" lignin was obtained from pine sawdust following the procedure described by Brauns (1).

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THE CAUSAL AGENTS OF THE BLACK STEM DISEASE OF ANNUAL LARKSPUR

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Summary

Erwinia atroseptica and E. chrysanthemi may cause a black stem disease of the annual larkspur. E. aroideae and E. carotovora may cause a green wet rot of young succulent larkspur plants.

Chester (2) and Ark et al. (1) in their separate investigations on the bacterial black stem of annual larkspur (Delphinium ajacis L.) attributed the cause of the disease to Erwinia atroseptica (van Hall) Jen. (E. phytophthora (Appel) Holland). Chester, however, states that there were some differences between the larkspur pathogen he was investigating and two isolates of E. atroseptica, one of which was tested for pathogenicity on potato stems. Ark et al. found cultural differences between the bacteria they had isolated from larkspur. In both investigations inoculation experiments proved the pathogenicity of their isolates on larkspur, and the descriptions of the external symptoms of the disease they produced were similar. Both investigators agree that the cortex was the tissue principally invaded but Chester states that the vascular bundles remained intact. Ark et al. states that this tissue eventually becomes invaded.

Neither Chester nor Ark report that they conducted inoculation experiments with the larkspur pathogens on potato stems to determine whether or not they would cause typical blackleg. Waldee (3) received from Ark a culture of a bacterium that the latter had isolated from diseased larkspur. The description of this isolate, Waldee states, followed in general that of E. atroseptica but differed from all the soft rot Erwinias in that it did not grow in Koser's citrate medium. He gave the single isolate the name, Pectobacterium delphinii.

If one examines the descriptions of these larkspur pathogens, there appears to be some confusion as to just what species the investigators had under consideration. The fact that Chester's bacterium produced acid in ethanol and that Ark's bacterium produced H_2S leaves one in doubt as to their correct determination. Neither of these characters are found in E. atroseptica although they may occur in other species of Erwinia. Methods of testing for the production of acid from ethanol and the production of H_2S in media vary, however, and interpretations can differ.

From the above review there appears to be a possibility that the black stem of larkspur may be caused by various species of Erwinia, and whether or not E. atroseptica is one of them is not certain. Inoculation experiments therefore were planned on young larkspur plants with a number of authentic isolates of various species in the genus Erwinia.

INOCULATION EXPERIMENTS AND RESULTS

The first set of experiments was carried out on a group of larkspur plants rather heterogeneous as to age and flower color. The method used was that of stabbing the plants both near the base and the tip of the stem with a sharp pointed scalpel contaminated with one of the bacterial species. The results of this set of experiments were as follows: of 10 plants inoculated with E. atroseptica, 2 showed typical black stem; of 6 inoculated with E. carotovora (Jones) Holland, 1 young succulent plant showed a green wet rot; of 6 plants inoculated with E. aroideae (Towns.) Holland, none were infected; of 6 plants inoculated with E. chrysanthemi Burk. et al., 3 showed typical infection; of 4 plants inoculated with a species of Erwinia isolated from carnation, 3 were infected; and of 2 plants inoculated with an Erwinia species from Philodendron, 1 was infected. The last two bacterial pathogens were similar to E. chrysanthemi and might have been this species, and both produced the black stem disease. In this series of experiments the purple flowered larkspur showed little or no infection.

Inoculations also were made on a similar group of larkspur by pouring water suspensions of the above bacteria about the roots of the plants without injuring the plant tissue. No infection resulted.

A further series of experiments were carried out on a group of white flowering larkspur plants of the same age. Plants 3 to 4 inches tall were inoculated in the same manner as in the first series of experiments. Of 4 plants inoculated with E. atroseptica 2 became infected; of

4 inoculated with E. aroideae, 1 succulent plant showed a green wet rot; of 4 inoculated with E. carotovora, 2 succulent plants died of a green wet rot; of 4 inoculated with E. chrysanthemi, 3 showed typical black stem; of 4 inoculated with the species from carnation, 2 showed long black to brown stem lesions, which is typical; of 2 plants inoculated with Erwinia from Philodendron, 1 died of a green wet rot; and of 2 plants inoculated with an Erwinia from corn (received from A. Kelman) both showed typical lesions except that the color was more reddish-brown than black.

A further set of similar experiments was conducted on mature white larkspur plants that had begun to flower. No typical black stem was produced but slight brown to black lesions developed on certain plants that had been inoculated with E. atroseptica and E. chrysanthemi.

DISCUSSION

From these inoculation experiments, all conducted in the greenhouse, it is deduced that E. atroseptica and E. chrysanthemi can cause a black stem disease of larkspur; and that E. aroideae and E. carotovora may infect young succulent larkspur plants and produce a green watery rot that results in the death of the plant. All of the bacteria used in these experiments were able to grow in Koser's citrate medium, therefore none could be identified as Waldee's P. delphinii. Since in no experiment was there 100 percent infection of inoculated plants, it appears that favorable conditions for the development of the disease were not present.

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TOXICITY OF OAK HEARTWOOD AND OAK HEARTWOOD WATER EXTRACTS TO THE OAK WILT FUNGUS[1]

James D. Bilbruck[2]

Abstract

The oak wilt fungus will not grow on the heartwood of red, black, northern pin, or bur oaks. A substance toxic to the oak wilt fungus, contained in the heartwood of these oaks, was found to be water-soluble, thermostable, non-volatile, and diffusible in agar-containing culture medium. Although tannic acid in a commercial preparation was toxic to the fungus at concentrations of 1 percent or more, apparently it was not the only substance in the extracts which may be toxic to the fungus. The bark supported fungus growth, but the heartwood did not; both contain an abundance of tannin. The toxic substance does not appear to be a resin, a pigment, such a quercetin, nor an alkaloid. Tests indicated that the toxic substance in the heartwood of these oak species may be tartaric acid. This acid is non-volatile, thermostable, soluble in water, and, in the pure state, toxic to the oak wilt fungus at concentrations of 0.6 percent and above, by weight, in water solution, even when mixed with wheat bran agar.

In tests conducted at the Illinois Natural History Survey from 1956 to 1958, the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt, would not grow on the heartwood of red (Fig. 1), black, northern pin, or bur oak (*Quercus borealis* Michx., *Q. velutina* Lamarck, *Q. ellipsoidalis* E. J. Hill, and *Q. macrocarpa* Michx., respectively).

Cross-sections of the wood of these species were moistened with 5 ml of water in Petri dishes and steam-sterilized for 1 hour; after cooling, the wood was inoculated by pouring 10 ml of a water suspension of oak wilt fungus conidia over the wood disk. Incubation was at 22° C, within the optimum range for oak wilt fungus growth (6, 11, 14).

When sawdust was prepared from the heartwood of these species of oak and autoclaved in water for 1 hour, the resulting filtered extract, mixed with equal parts of wheat bran agar, would not support oak wilt fungus growth. Bark and sapwood of these species, or extracts made from them, did support growth of the fungus.

The work reported in this paper was undertaken in an attempt to determine the nature and properties of the toxic substance or substances in oak heartwood which inhibits growth of *C. fagacearum*.

In a study of five naturally infected red oaks in Iowa, Young (22) never isolated the oak wilt fungus from the heartwood; he found that the radial distribution of the fungus was restricted to the outer quarter inch of sapwood. Henry and Riker (8) state that the radial distribution of the fungus in the stems extended to the 2-year-old summer wood in naturally-infected trees and to the 2-year-old spring wood in artificially-inoculated trees, in a relatively solid band of wood.

According to Wise (20), the following substances, which apparently are toxic to fungi, have been found in oak wood: tannins, quercetin, and the salts of oxalic, malic, and tartaric acids. The tannin in the wood is a hydrolyzable tannin, that in the bark is a phlobotannin. Hydrolyzable tannins occur in pathological growths, such as the gallotannin in oak leaf insect galls. There is some indication that phlobotannins contain catechol (20). Protocatechuic acid is a tannin derivative in oak bark (2). Apparently the effect of such materials is quite specific for each species of fungus (1, 3, 4, 5).

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[1] A portion of a thesis submitted to the University of Illinois, Urbana, Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology (1957). The work was done at the Illinois State Natural History Survey, Urbana.

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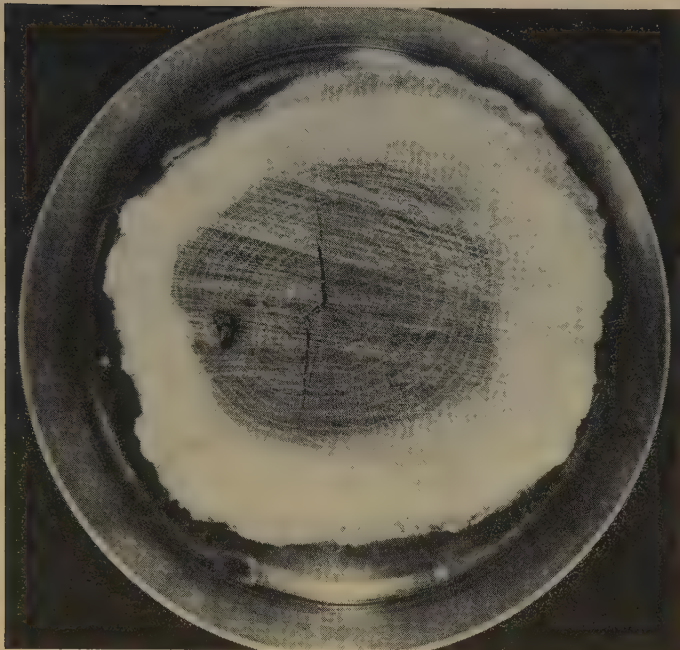

FIGURE 1. The oak wilt fungus growing on a disk of red oak, 5 3/4 inches in diameter and 1/2 inch thick. The fungus did not grow on the heartwood. Illinois State Natural History Survey photograph.

TOXICITY OF HEARTWOOD WATER EXTRACTS

As a standard proportion, 50 gm of oak heartwood sawdust to 100 ml of distilled water were used; the mixture was placed in Erlenmeyer flasks and autoclaved 1 hour. After filtering, the liquid portion was mixed in equal parts with wheat bran agar. Wheat bran agar was made by adding 75 gm of wheat bran to 750 ml of distilled water, boiling until starchy material was evident, and straining. After straining, the material was mixed with a 15-ml solution of Bacto-agar in 250 ml of distilled water and autoclaved 45 minutes. This culture medium, a good one for oak wilt fungus growth, was used to give as severe a test as possible to the toxic substance in the extracts.

The filtrate from the heartwood water extraction was a clear, amber to brown solution. Most of the tests for toxicity to the oak wilt fungus were made with heartwood water extract and wheat bran agar mixtures.

Dilution of the "standard mixture" of heartwood sawdust and water inhibited oak wilt fungus growth in the order of 20, 10, 5, and 2.5 ml of water extract to 20 ml, each, of wheat bran agar. A dilution of 1 ml of extract to 20 ml wheat bran agar did not. When the extract was concentrated by distillation of half the water, under vacuum, the result of this dilution series was the same as noted above. Similar dilutions of distillate-wheat bran agar mixtures supported oak wilt fungus growth in all cases. Wheat bran agar controls were inoculated with oak wilt fungus conidia in all tests reported in this paper.

pH OF OAK HEARTWOOD EXTRACTS AND EXTRACT-WHEAT BRAN AGAR MIXTURES

Hoffmann (11) determined the optimum range for oak wilt fungus growth on solid substrates to be from pH 5.0 to 7.0. To study the influence of pH on the toxicity of the extracts to the fungus, pH determinations were made with a Beckman glass electrode pH meter. A list of pH readings follows. Wheat bran agar has a pH of 6.1.

pH of Extracts and Extract-agar Mixtures

	Heartwood water extract	Extract plus wheat bran agar
Red Oak	pH 3.8	pH 4.3
Black Oak	3.5	5.6
Bur Oak	---	5.0
Northern Pin Oak	---	4.8

pH of Several Dilutions of Extracts with Wheat Bran Agar

Extract		Bur Oak	Red Oak Concentrate	Red Oak Distillate
20.0 ml plus	20 ml wheat bran agar	pH 4.7	pH 4.0	pH 5.2
10.0	do.	5.1	4.4	5.5
5.0	do.	5.6	4.5	5.7
2.5	do.	5.8	5.3	5.9
1.0	do.	--	5.7	6.0

All extract-agar mixtures and extract-concentrate-agar mixtures inhibited growth of the fungus, with the exception of the 2.5 to 20 ml bur oak heartwood extract-wheat bran agar and the 1.0 to 20 ml red oak heartwood extract concentrate-wheat bran agar mixtures. The distillate-wheat bran agar mixtures of the red oak heartwood extract did not inhibit growth of the fungus at any dilution.

Adjustment of the pH of red and black oak heartwood water extracts was accomplished by adding 10 percent aqueous potassium hydroxide solution until a pH between 5.5 and 7.0 was reached. When extract-wheat bran agar mixtures were made, after this pH adjustment, all inhibited growth of the fungus.

ACTION OF ORGANIC SOLVENTS ON THE WATER EXTRACTS

As a step toward determining the solubility of the toxic substance in the water extracts, organic solvents were mixed with equal portions of the extracts. The solvents were chloroform, benzene, toluene, carbon tetrachloride, t-butyl alcohol, xylene, isoamyl alcohol, isopropyl alcohol, cyclohexanone, and diethyl ether.

Apparently the toxic substance was soluble in none of the solvents. Chloroform, benzene, toluene, and t-butyl alcohol caused precipitation in the extracts, but the supernatant (concentrate, in the case of t-butyl alcohol) inhibited oak wilt fungus growth in all cases. Traces of solvent were driven off by heating after decanting and the supernatant mixed with wheat bran agar. The other solvents caused little or no precipitation.

Mixtures of carbon tetrachloride, chloroform, benzene, and toluene were made with equal parts of distilled water. After the solvent had been driven off in each case, the remaining water was mixed with wheat bran agar and tested for toxicity to the fungus. Only carbon tetrachloride left a residue toxic to the fungus in the water. Chloroform was the most useful of these solvents. It was used subsequently to remove gummy, mucilaginous, and proteinaceous material from the extracts.

TANNIC ACID

Tannic acid (tannin) has been found toxic to many fungi (3, 4, 5, 7, 15). Several tannic acid-distilled water dilutions were mixed with equal parts of wheat bran agar and tested for toxicity to the oak wilt fungus. Dilutions of 1 percent or greater inhibited growth.

When tannic acid was precipitated from the extracts with gelatin-alcohol solution, the supernatant inhibited growth of the oak wilt fungus.

PRECIPITATION INDUCED BY CHEMICALS

Portions of black oak heartwood water extract were tested with aqueous solutions of several chemicals, according to directions for the analysis of plant water extracts given by Wittstein (21). The chemical solutions, measured by weight, are as follows: Ammonium hydroxide, 10%; potassium hydroxide, 34%; potassium carbonate, 10%; barium oxide, 5%; limewater (1 gm CaO to 700 ml distilled water); ammonium chloride, 10%; calcium chloride, 10%; calcium acetate, 10%; ferric chloride, 10%; ferrous sulfate, 10%; lead acetate, 10%; antimony and potassium tartrate, 5%. The solutions were added drop by drop to separate 15-ml portions of the extract. Since these chemicals precipitate different organic substances from water solutions, the object of the series of tests was to determine which chemical would leave the supernatant non-toxic to the oak wilt fungus, thus giving an indication of what the toxic substance in the extract might be.

Barium oxide produced a brown, flocculent precipitate. According to Wittstein (21), this indicates the presence of alkaloids minus their acid component, sulfuric or phosphoric acids, organic acids, or pigments acting as acids. Alkaloids were not considered, since Henry (9),

in his book on plant alkaloids, does not list any member of the family Fagaceae as containing them. Further treatment with barium chloride produced no precipitate, indicating the absence of hydrochloric, sulfuric, or phosphoric acids. The supernatant from the barium oxide treatment was the only one that supported oak wilt fungus growth. The precipitate from this treatment was also non-toxic to the fungus. This indicates that barium oxide precipitates and inactivates the toxic substance in the extract.

Limewater produced a granular brown precipitate, indicating the presence of tartaric acid. A further indication of the presence of tartaric acid was secured by treatment of the limewater-extract mixture, containing the precipitate, with ammonium chloride; the precipitate was redissolved.

Lead acetate produced an abundance of dirty brown precipitate. The precipitate was redissolved when 10 percent acetic acid was added, indicating the presence of organic acids, pigments, protein-substances, and resins. Resins were not considered, since they are not known to occur in oak (20). Since protein substances were precipitated by chloroform without reducing the toxicity of red or black oak heartwood water extracts to the oak wilt fungus, it seems reasonable to consider these substances as non-toxic to the fungus.

Barium chloride, calcium chloride, calcium acetate, and ferrous sulfate also produced precipitates in the extracts; the other chemicals which have not been discussed did not.

The use of lead acetate was discontinued when testing extracts to be used later for growth tests of the oak wilt fungus, since non-fat liquid milk and distilled water, when treated with lead acetate and the lead precipitated with hydrogen sulfide, filtered and freed of hydrogen sulfide by heating, proved to be toxic to the fungus.

QUERCETIN AND QUERCITRIN

Since precipitation with barium oxide indicated the presence of pigments (21), commercial preparations of high purity of the pigments present in oak wood were tested for toxicity to the oak wilt fungus. These pigments are quercetin and its rhamnoside derivative, quercitrin (2, 10, 13, 16, 17, 21). Neither inhibited growth of the fungus, when mixed with wheat bran agar, even when a saturated solution or a suspension was used.

NON-VOLATILE ORGANIC ACIDS

Since the water extracts could be concentrated by distillation without loss of toxicity and the distillate itself was non-toxic to the oak wilt fungus, the toxic substance contained therein apparently was not one of the volatile organic acids, such as acetic acid. The precipitation with barium oxide left one other alternative to be investigated, namely, testing for the presence of non-volatile organic acids.

Acids in black oak heartwood sawdust were extracted by methods listed by Klein (12), that is, by successive treatment of the sawdust with petroleum ether, anhydrous diethyl ether, 95 percent ethanol, cold distilled water, warm distilled water, and 5 percent aqueous hydrochloric acid. Filtering was done between each treatment. The sawdust was allowed to dry between treatments, except the water and hydrochloric acid.

The alcohol and hydrochloric acid filtrates, which are most likely to contain acids (12), were tested for the presence of oxalic, tartaric, and malic acids.

The alcohol filtrate was distilled under vacuum. The concentrate was treated with lead acetate and hydrogen sulfide, then filtered. The filtrate was treated with ammonium and calcium chloride solutions. A precipitate appeared, indicating the presence of tartaric acid.

When a portion of the filtrate from the heartwood sawdust treatment with hydrochloric acid was treated with calcium and ammonium chlorides, a precipitate appeared, again indicating the presence of tartaric acid. Another portion of the filtrate from the treatment with hydrochloric acid was subjected to the resorcinol-sulfuric acid test cited by Klein (12). A definite red ring, at the interface of the resorcinol-extract solution and the concentrated sulfuric acid placed together in a test tube without mixing, showed the presence of tartaric acid. The blue ring for oxalic acid failed to appear. None of the tests for oxalic or malic acids indicated their presence. When the filtrate was treated with barium acetate no precipitation occurred, indicating the absence of citric acid.

Tests of mesotartaric acid for toxicity to the oak wilt fungus showed that this acid is toxic to the fungus at concentrations of 0.6 percent or above, when mixed with equal parts of wheat bran agar. Wertheim (19) states that the chemical properties of all isomers of tartaric acid are similar.

DISCUSSION AND CONCLUSIONS

Failure of the oak wilt fungus to grow on heartwood of these oak species or in culture media containing water extracts made therefrom indicates the presence of a substance that is toxic to the fungus.

Thermostability of the toxic substance is indicated, since autoclaving for 1 hour of wood pieces or water extracts did not destroy its toxicity. The diffusibility of the toxic substance in wheat bran agar and the toxicity of filtered water extracts from oak heartwood indicate solubility of the toxic substance in water. The non-volatility of the toxic substance is indicated, since it always remained in the concentrate when extracts were distilled; this rules out volatile substances such as acetic acid.

Low pH apparently does not explain toxicity of the heartwood extracts to the oak wilt fungus, since the pH of the extract-wheat bran agar mixtures was within or close to the optimum for growth of the fungus (11).

Precipitation of tannic acid did not alter toxicity of the heartwood extracts to the oak wilt fungus. Furthermore, the fungus grows on oak bark, which contains an abundance of phlobotannin (7, 20). Since the tannin in the heartwood is a hydrolyzable tannin (20), this difference may explain the non-toxicity of bark and extracts made therefrom, but it does not explain the toxicity of heartwood extracts from which tannin has been precipitated.

The test with barium oxide narrows the possible toxins to organic acids, disregarding substances not known to be present in oak wood and those, such as pigments, found to be non-toxic to the oak wilt fungus. Non-volatility of the toxic substance further limits the possible toxins to non-volatile organic acids. Tartaric acid was the only organic acid indicated by this series of tests. Further, mesotartaric acid was found to be toxic to the fungus at 0.6 percent or higher concentrations. Results of these tests indicate that tartaric acid may be an important factor in preventing growth of the oak wilt fungus in the heartwood of black, red, northern pin, and bur oak. However, since pure tartaric acid was not obtained from the heartwood extracts, its presence is indicated, but not definitely established, in the extracts.

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TRUNK LESION OF SWEETGUM

E. Richard Toole and R. C. Morris[1]

In 1957, John A. Putnam of the Southern Forest Experiment Station called to our attention a trunk lesion of sweetgum (Liquidambar styraciflua L.). He had observed this defect over a number of years in bottom-land stands within about 100 miles of the Gulf Coast.

While the defect does not kill trees, it degrades lumber and veneer. This paper describes the lesion, gives 1-year results from observations of sample trees, and summarizes early tests that point to a fungus as the primary cause.

THE LESIONS

The lesions result from the killing of limited patches of cambium. The first evidence is a small spot of fresh storax or gum oozing from the bark. Later a crack appears, and the gum flowing down the trunk blackens and hardens (Fig. 1A, 1B). Frequently oozing cracks are grouped around older healed areas. Pronounced bumps or ridges are formed by the rapid

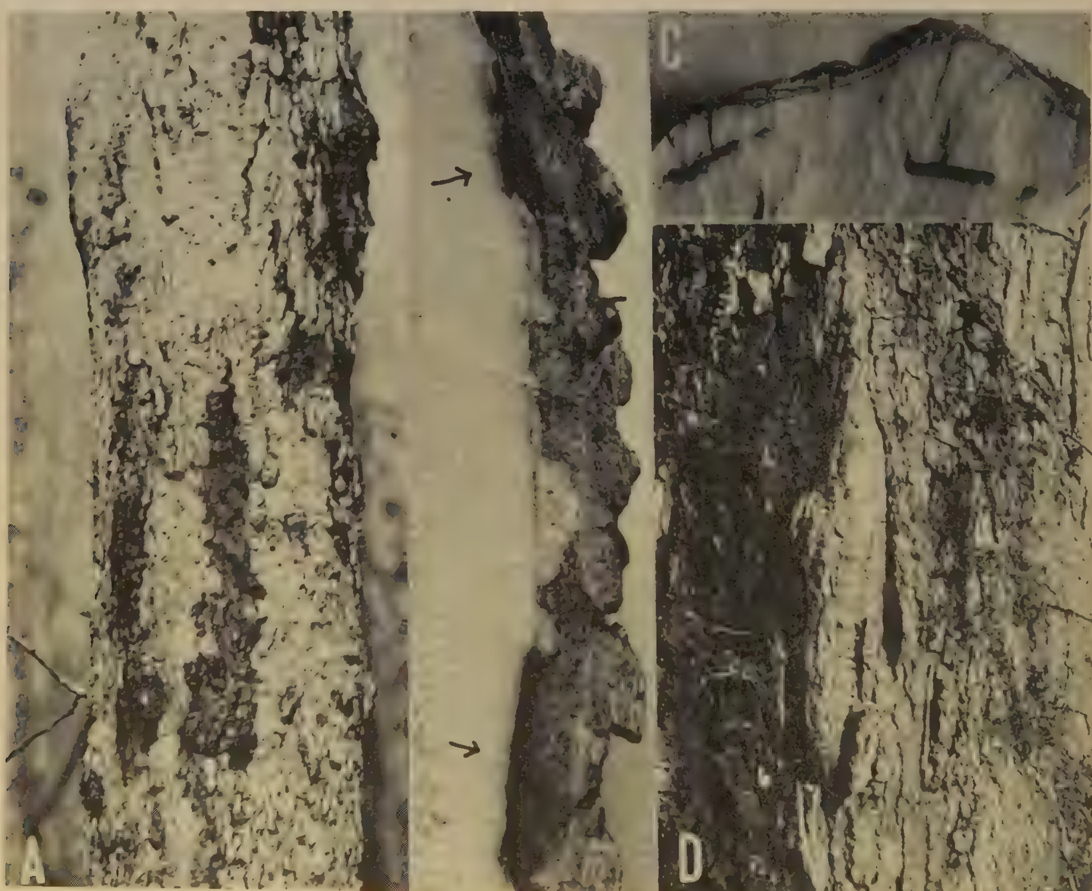

FIGURE 1. Trunk lesions on sweetgum: A -- A young lesion less than a year old; B -- Cross section through diseased bark. Arrows indicate areas where the cambium has been killed; C -- Cross section through a 6-year-old inactive lesion. A small strip of cambium was killed and the callus tissue formed during healing produced the "bump" or ridge in the wood; D -- A lesion about 10 years old and inactive.

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[1] The authors are members of the Southern Forest Experiment Station, Forest Service, United States Department of Agriculture. They are stationed at the Stoneville Research Center, which is maintained at Stoneville, Mississippi, in cooperation with the Mississippi Agricultural Experiment Station and is partially financed by the Southern Hardwood Forest Research Group.

FIGURE 2. Small sweetgum lesion: A -- from the outside; B -- with outer bark removed to show discolored phloem; C -- all bark removed to show extent of dead cambium.

growth of callus tissue as the lesions heal (Fig. 1C). The phloem is killed in irregular patches, sometimes as deep as the cambium. The area of discolored phloem is always larger than the area of dead cambium (Fig. 2). Sections through the ridges on the trunks disclose small discolored lines in the wood, formed when patches of killed cambium callused over (Fig. 1D). In some areas of the infected stem lesions are formed year after year.

RANGE AND DISTRIBUTION

Detailed surveys have not been made, but the disease has been found in Texas in the bottoms of the Saline, Neches, Angelina, Trinity, and San Jacinto Rivers; in Louisiana along the Calcasieu River and in other bottom-land locations; in Mississippi along Bayou Pierre and the lower Pearl, Big Black, and Leaf Rivers; in Alabama along the lower Alabama, Tombigbee, Escambia, and Conecuh Rivers; and in west Florida in the Chipola, Apalachicola, Ochlokonee, and Chattahoochee bottoms. It has been found along small as well as large streams and on a wide variety of soils, but so far only on bottom-land sites.

Active sweetgum lesions are most common in stands 10 to 20 years old. Occurrence in any area is patchy. Sometimes 25 to 50 percent of the sweetgums on an acre will be infected, while nearby trees will be free. In older stands, the ridged effect is more pronounced. The lesions are found on all sides of the tree and from the groundline to a height of 25 feet. They are most common on the lower 8 feet of stem.

STUDY PLOTS

To obtain data on the progress of the lesions, plots were established in early 1958 in Clarke County, Alabama, Perry County, Mississippi, and Allen Parish, Louisiana -- three plots in each place. The Alabama plots are in a pure old-field stand on the bank of the Alabama River; on two plots trees were about 12 years old, on a third about 50 years. The Mississippi plots are along the Leaf River, near Beaumont, in mixed hardwood stands where the sweetgums are 50 to 60 years of age. The plots in Louisiana are along Tanny Creek, southeast of Oakdale, in 20-year-old stands.

Of the trees that were healthy in February 1958, an average of 8 percent had developed lesions by 1959 (Table 1). Of those with lesions at the start, 39 percent had additional infections after 1 year (Table 2). Twice as many new lesions were formed between April and September as from October through March. Within these periods there was no indication that

Table 1. Progress of lesions on sweetgum trees that were healthy in February 1958.

Location and plot number	Healthy in 1958	Diseased by February 1959
	- - - Number of trees	- - -
Clarke County, Alabama		
1	10	2
2	8	1
3	8	3
Allen Parish, Louisiana		
4	22	1
5	23	2
6	22	0
Perry County, Mississippi		
7	21	1
8	17	1
9	8	0
Total	139	11

Table 2. Progress of lesions on sweetgum trees that were diseased in February 1958

Location and plot number	Diseased in 1958	Worse by February 1959
	- - - Number of trees	- - -
Clarke County, Alabama		
1	20	13
2	21	13
3	22	7
Allen Parish, Louisiana		
4	8	1
5	7	1
6	8	2
Perry County, Mississippi		
7	9	4
8	3	2
9	22	4
Total	120	47

Table 3. Proportion of infected wounds 3 and 9 months after inoculation with tissue and fungal cultures from lesioned sweetgum trees

Inoculum	Clarke County, Alabama		Perry County, Mississippi		Allen Parish, Louisiana		Average	
	After 3 mo.	After 9 mo.	After 3 mo.	After 9 mo.	After 3 mo.	After 9 mo.	After 3 mo.	After 9 mo.
				- - - - Percent	- - - -			
None	0	0	0	0	0	0	0	0
Diseased tissue	30	50	10	20	30	10	23	27
Fungus 1	[a] 10	0	[a] 50	0	[a] 20	0	[a] 27	0
Fungus 2	0	0	0	0	0	0	0	0
Fungus 3	60	100	50	80	70	80	60	87
Fungus 4	0	0	0	0	[a] 10	[b] 10	[a] 3	[b] 3
Fungus 5	[a] 40	[b] 10	0	0	[a] 40	0	27	[b] 3
Fungus 6	[a] 70	[b] 10	[a] 10	0	[a] 10	0	[a] 30	[b] 3

[a] Slight infection with little sap flow.

[b] Wounds appear healed, but are exuding some gum.

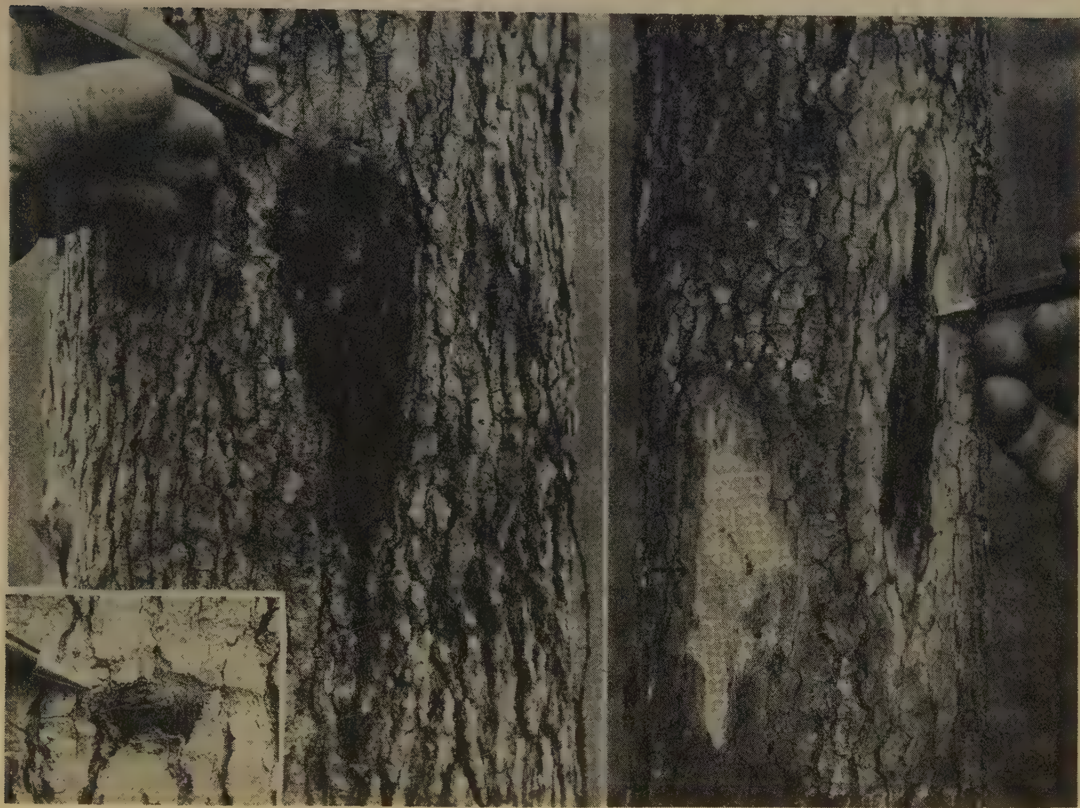

FIGURE 3. Left -- Typical lesion produced by fungus 3, 9 months after inoculation. Insert at lower left shows a healed check inoculation point. Right -- Outer bark removed from inoculation points 9 months after inoculation. Lesion on right was produced by fungus 3;pencil indicates point of inoculation. Point by arrow was inoculated with a non-pathogenic fungus.

one time was more favorable for lesion production than another. Lesions tended to dry up as they aged, but dried lesions commonly oozed gum again from time to time.

SEARCH FOR CAUSE

No evidence was found to indicate that the defect is caused by insects, but several unidentified fungi were isolated.

In May 1958, inoculations were made on ten trees near the plots in the three States -- 30 trees in all. On each tree, eight wounds made with a sterilized knife were spaced spirally around the bole from 3 to 6 feet above the ground. One hole on each tree was inoculated with sterile agar, one with diseased tissue, and one with each of the six fungi commonly isolated from lesions.

After 3 months (Table 3) definite lesions appeared at some of the points inoculated with diseased tissue and with fungus No. 3. Slight infection was evident with some of the other fungi. Six months later (9 months after inoculation), 87 percent of the wounds inoculated with fungus No. 3 and 27 percent of those inoculated with diseased tissue were still open; all the rest had healed. Figure 3 illustrates typical results of inoculation.

The inoculations with diseased tissue showed that lesions are infectious, and the results with fungus isolate No. 3 strongly suggest that it is the causal agent. This fungus has not produced spores in culture, and therefore cannot as yet be identified.

UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST SERVICE, SOUTHERN FOREST EXPERIMENT STATION

A SIMPLIFIED METHOD FOR FIELD INOCULATION OF SOYBEANS WITH BACTERIA

John P. Jones and Edgar E. Hartwig[1]

Field evaluation of soybean lines for reaction to bacterial pustule (Xanthomonas phaseoli var. sojense (Hedges) Starr & Burkholder) has been materially simplified under conditions at Stoneville, Mississippi, through the use of inoculum prepared from infected soybean leaflets.

Field results obtained during the past 3 seasons have shown that excellent infection could be obtained by using fresh X. phaseoli var. sojense-infected leaflets as a source of inoculum. Satisfactory infection has been secured with an inoculum concentration of 10 infected leaflets per gallon of water. A gallon of inoculum is prepared by passing 10 freshly-picked, moderately-infected leaflets through a household food chopper, and then comminuting this material in 300 to 600 ml of tap water in a food blender. The suspension is allowed to stand for 1 to 2 hours to permit bacterial diffusion from the leaf fragments, then filtered through two layers of cheesecloth and brought to 1 gallon with tap water. The leaflet-inoculum is then employed in the same manner as pure-culture inoculum.

Leaflet-inoculum has been applied by low- and high-pressure sprayers, with equally satisfactory results. Ordinarily small-plot inoculations are made with knapsack sprayers and large-scale tests with tractor-mounted equipment.

Inoculation tests of susceptible soybean plants in the greenhouse and field showed that pustule-infected leaflets could be stored from season to season for use as inoculum. Infected leaflets are minced in a food chopper, placed into glass fruit jars which are then capped, and stored at 0° to 20° F. The frozen material is used in the preparation of inoculum in the same manner as fresh leaflets. One hundred to 150 cc of frozen material per gallon of water gives satisfactory infection in the field. Infected leaflets have been stored in this manner for 23 months without any apparent diminution of bacterial pathogenicity. When tested after 30 months storage, however, infectivity was found to be greatly reduced. Consequently, yearly or biyearly replacement of stored inoculum would appear advisable.

The leaflet-inoculum technique is used at Stoneville in the following manner: Soybeans are ordinarily planted at this station during May. Plantings made in mid-May are at a satisfactory stage for inoculation by mid-June. In order to obtain infected fresh leaf material for inoculum by mid-June, a small plot of a pustule-susceptible soybean line is planted in mid-April. The plants of this line are inoculated when they are 4 to 6 weeks old. The inoculum is prepared from frozen, infected-leaf material stored from the previous season. The resulting infected leaflets in this "inoculum garden" provide a large volume of virulent inoculum over an extended period, which permits the inoculation of experimental lines at any time conditions are favorable. This feature eliminates the sometimes critical timing necessary with pure-culture inoculum. At the same time that fresh leaflets are picked for inoculum others are collected and frozen for use the following season.

The method has several advantages, the most obvious being simplicity resulting from the elimination of a large amount of tedious laboratory culture work. Routine inoculation procedures can easily be handled by technicians. Virulence loss or strain selection that can occur in pure culture is greatly reduced. Furthermore, the method more nearly approximates natural infection and therefore gives a better field evaluation of the soybean lines under test.

Similar results have been obtained through 2 seasons with the soybean wildfire organism, Pseudomonas tabaci (Wolf & Foster) F. L. Stevens. Tests have also shown that wildfire-infected leaflets can be stored frozen for over 2 years without diminution of infectivity.

The inoculation technique described can very likely be adapted for routine field use with other leaf-infecting bacteria.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE; AND DELTA BRANCH OF THE MISSISSIPPI AGRICULTURAL EXPERIMENT STATION, STONEVILLE, MISSISSIPPI

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NEW OR UNUSUAL OCCURRENCE OF CERTAIN DISEASES IN LOUISIANA

J. B. Sinclair, N. L. Horn and W. J. Martin[1]

SEPTORIA LEAF SPOT OF TOMATO

Although departmental records did not show Septoria lycopersici to have occurred in Louisiana before, Dr. A. G. Plakidas, Louisiana State University Experiment Station, had observed it occasionally on fall tomatoes. However, this is the first time that Septoria leaf spot has been found on tomatoes in the spring crop. The disease was found in West Carroll Parish of northeast Louisiana near the Arkansas border during the latter part of May. It is only the second year that tomatoes have been grown in this area for commercial production. The foliage spot was found scattered in about one-third of the 200 to 250 acres planted. Defoliation of maturing plants had occurred up to about the third cluster of fruit. The disease was most severe in plantings where the grower did not or could not follow the recommended spray schedule because of weather conditions.

LEAF MOLD OF TOMATO

Cladosporium fulvum on tomato has been observed intermittently in Louisiana by several plant pathologists since 1922, either on greenhouse grown plants or on field plants late in the fall. The infection had been light and the damage small, so that formal reports have never been made. This year the disease took on new importance when it occurred for the first time on spring tomatoes in the field. The first report came from Franklin Parish near Winnsboro in northeast Louisiana on June 4. The area affected was large enough and severe enough to cause the grower some concern. In an acre field of tomatoes about 50 to 75 plants were killed by the fungus. The disease was reported again on June 8 on tomatoes in experimental plots on the Louisiana State University campus. The damage here was slight.

WEATHER CONDITIONS

Weather conditions were important factors in the occurrence of both Septoria leaf spot and leaf mold of tomato in the field. Favorable temperatures for disease development, frequent rains and high humidity were common in these areas during the latter part of May and the first part of June.

TABASCO PEPPER WILT

The growing of Tabasco pepper (Capsicum sp.) is limited to three parishes in south Louisiana. Considerable losses due to a wilting disease have been experienced by growers in these areas for many years. Departmental research files show that the disease was recorded for the first time in 1935, and at that time various fungi isolated from wilted plants did not reproduce the disease under controlled conditions. In 1953, it was shown that the tobacco-etch virus (TEV) was the cause of a Tabasco pepper wilt disease[2]. Greenhouse and field studies during the past year have confirmed that Tabasco pepper wilt in Louisiana is incited by TEV. This is the first published report of the disease and its cause for Louisiana.

LEAF SPOT OF RED LASODA IRISH POTATOES

A very severe leaf spotting on Red LaSoda Irish potatoes was first noticed by Dr. W. J. Martin in 1952 and has occurred each year since then in the Irish potato-growing areas of Louisiana. The disease usually appears about half way through the growing season and severely affected plants are yellow, stunted and generally unthrifty. The numerous leaf spots are small, somewhat circular, black to brown on the upper surface of the leaf and black on the under surface (Fig. 1). The symptoms look like an early stage of early blight, but Alternaria

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[2]Greenleaf, W. H. 1953. Effects of tobacco-etch virus on peppers (Capsicum sp.). Phytopathology 43:564-570.

FIGURE 1. Leaf spot of Red LaSoda Irish potatoes. Both young and older leaves are affected. Upper surfaces of diseased leaves are shown on the right and upper left, lower surfaces of diseased leaves in center and lower left.

solani spores have not been found on diseased leaves after repeated examinations. Isolations from leaf spots yielded Alternaria- and Colletotrichum-like organisms in culture, but these did not prove to be pathogenic when reinoculated to healthy plants. Observations in 1958 and 1959 indicate that the disease may be a deficiency symptom occurring when the recommended fertilization program is not followed.

LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE

UNUSUAL RECORDS OF PLANT DISEASE OCCURRENCE

UNUSUAL OCCURRENCE OF TOMATO LEAF MOLD IN LOUISIANA

By J. B. Sinclair

An earlier report (PDR, this issue, page 947) this year cites the unusual occurrence of Cladosporium fulvum on spring tomatoes growing in two different areas. Since this report was made, diseased tomato specimens, either sent into or brought into this laboratory, indicate that leaf mold reached epidemic proportions throughout Louisiana. Both commercial and home garden plantings were severely affected and one field may have had severe losses due to a fruit rot possibly caused by the same organism.

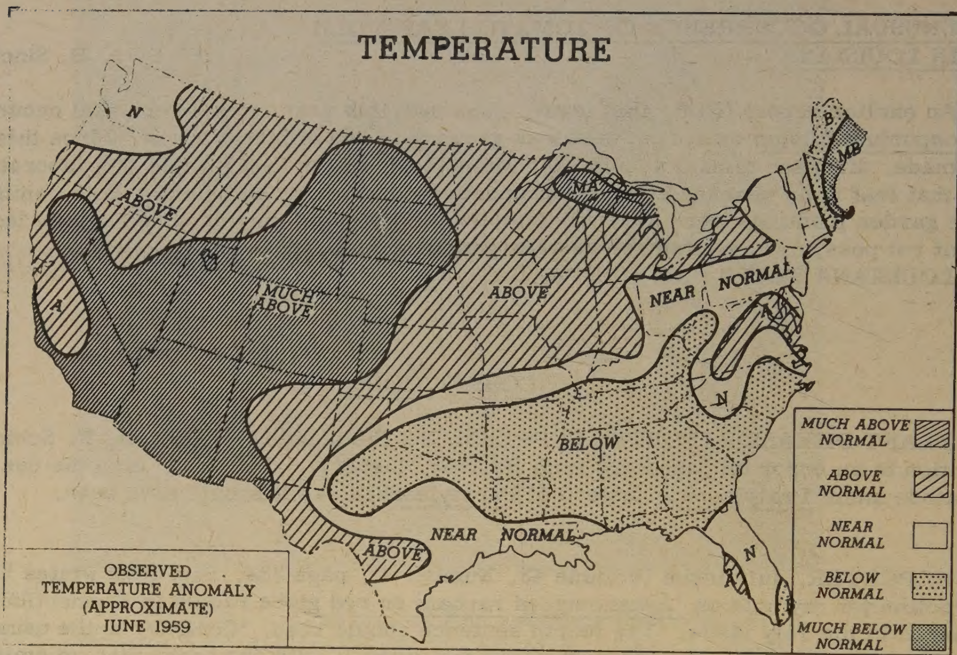
LOUISIANA STATE UNIVERSITY, BATON ROUGE, LOUISIANA

CORRECTIONS

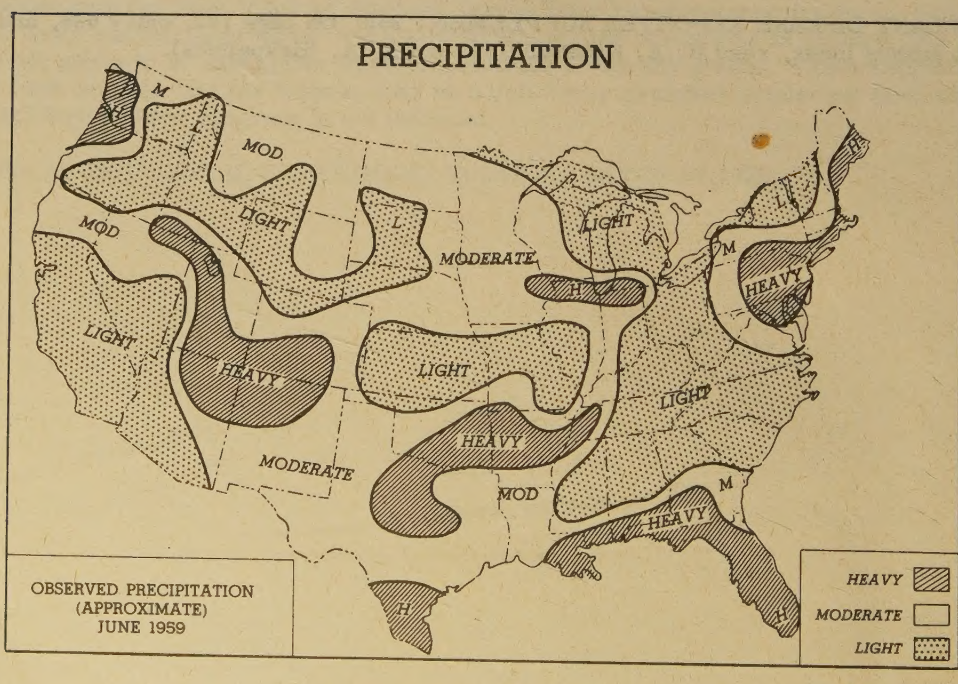
PLANT DISEASE REPORTER SUPPLEMENT 256 (June 15, 1959): A. F. Schindler calls attention to an error on page 141, Item No. 548, last line. The error is in the use of the nematode name Pratylenchus instead of Paratylenchus, as it should have been.

REPORTER, July issue (Volume 43, Number 7), page 834: Saul Rich writes "Here is a correction for my note on 'Aphanomyces raphani on red globe radish in Connecticut', which appeared in the July issue. The fourth sentence should read, 'Contrary to the usual symptom picture, there were few lesions on the long tap roots.'" We are sorry that we omitted the key word few from this report.

PLANT DISEASE REPORTER SUPPLEMENT 256: On page 162, entry 698, and page 172 in the author index, read R. A. KILPATRICK (not R. A. Kirkpatrick).



OBSERVED TEMPERATURE ANOMALY (APPROXIMATE) JUNE 1959



OBSERVED PRECIPITATION (APPROXIMATE) JUNE 1959

The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.